HOST DISTRIBUTION AND PHYSIOLOGICAL EFFECTS OF ECTOCOMMENSAL GILL BARNACLE (*Octolasmis muelleri*) INFESTATION ON BLUE CRABS (*Callinectes sapidus*).

By

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This work is dedicated to my partner
and wife,
Francesca.

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The relationship between an ectocommensal gill barnacle, Octolasmis muelleri, and its host, the blue crab (Callinectes sapidus), was studied on three levels: population, organism and model for host/commensal systems. On the population level, barnacle distribution was evaluated in the Cedar Key, Florida blue crab population and within the host gill chamber. Of 529 crabs, 40% were infested, with monthly infestation rates ranging from 17% to 67%. The mean number of barnacles per infested crab was 21.8 with as many as 432 barnacles on one crab. The barnacles aggregated on their expected optimal host, namely previously infested adult blue crabs. They were also most frequent at their expected optimal site

within the host gill chamber, namely the base of the hypobranchial side of gills 3, 4, 5 and 6. This distribution indicates that O. muelleri infestation is common and frequently heavy and suggests that host and site-within-host selection occurs. At the organism level, infested blue crabs compensate for the obstruction in their ventilatory stream by increasing ventilation (1.8 X) and heart rates (1.4 X) to maintain oxygen consumption at the same level (28 μmol kg^{-1} min^{-1}) as uninfested crabs. Moderately infested crabs (10-20 ectocommensals) had small differences in hemolymph pH, oxygen tension and oxygen content. During exercise most differences between infested and uninfested crabs disappeared. Greater arteriovenous differences in hemolymph pH and lactate levels of infested crabs suggest that they have a greater reliance on the Bohr effect for oxygen delivery to the tissues, but hemocyanin-O_2 affinity is preserved at the gills by a counteracting lactate effect. At the host/ectocommensal level, the barnacle has undergone selection to choose optimal hosts and attachment sites, and to minimize detrimental effects to the host. The blue crab has undergone selection to compensate for environmental stresses, such as barnacle infestation. In all but extremely heavy infestations the barnacle has a minor effect on host crabs at rest, during sustained swimming and during recovery.

GENERAL INTRODUCTION

The blue crab, Callinectes sapidus Rathbun (1896), is a widely distributed and well-studied swimming crab (Portunidae). Population levels are large enough to support commercial fisheries along the Gulf coast and the Eastern seaboard of the United States. By nature Callinectes is a very active crab, and a high rate of respiratory gas exchange is central to its metabolism and growth. Because this crab is relatively long-lived (up to three years as an adult; Van Engel, 1958), moves long distances (Oesterling, 1982) and provides a hard substratum in soft bottom estuaries (Williams, 1984), it serves as host to numerous obligate and accidental commensals, such as the soft coral Leptogorgia virgulata (Pearse, 1947), the bryozoan, Triticella elongata (Maturo, 1957), the leech Myzobdella lugubris (Overstreet, 1982), the sessile barnacle, Chelonibia patula, (Van Engel, 1958), the oyster, Crassostrea virginica, (personal observation) and the tunicate Molgula manhattensis (Pearse, 1947).

Callinectes sapidus is the favored host of Octolasmis muelleri (Coker, 1902), an ectocommensal stalked barnacle found

on the gills or in the gill chamber of decapod crustaceans (Humes, 1941). My aim was to examine this host/ectocommensal relationship. Initially, the distribution of O. muelleri within the Gulf coast blue crab population and within the blue crab gill chamber was investigated. After characterization of the barnacle distribution, the effects of O. muelleri infestation on the respiratory physiology of the blue crab host were measured. Finally, the effects of O. muelleri infestation on the respiratory physiology of blue crabs under the natural stress of swimming were measured. Determination of the magnitude and ways that blue crab respiration is affected by barnacle infestation is important in assessing the potential threat to an important commercial resource, understanding the mechanisms by which the crab (a model organism for crustacean respiration studies) compensates for barnacle infestation, and evaluating the benefits and detriments of the host/ectocommensal relationship.

Blue Crab Respiration

The anatomy and physiology of the respiratory and circulatory systems of the decapod crustaceans have recently been reviewed by McLaughlin (1983) and McMahon and Wilkens (1983). In summary, blue crabs possess eight gills on each side of the body. The gills are phyllobranch, triangular, wide at the base and narrowing distally as they arch dorso-posteriomedially. The gills are housed in the branchial chamber, which is formed as a lateral outgrowth of

the carapace. The carapace curves under to fit tightly against the lower body wall just above the insertion of the walking appendages. This joint is tight enough to exclude water. Water flows into the gill chamber primarily through the Milne-Edwards inhalent opening at the base of the cheliped. Smaller inhalent holes are found at the bases of the pereiopods.

As water enters the Milne-Edwards hole it flows posteriorly. It first enters the branchial chamber under the gills in the hypobranchial chamber between gills 3 and 4 (Aldridge and Cameron, 1982). From there the water flows through the gills and, once it enters the epibranchial chamber, it flows anteriorly exiting the branchial chamber via the exhalent passage, which is formed by the endo- and exopodites of the three maxillipeds. The propulsive force for water movement is the generation of negative pressure in the branchial chamber by the beat of the scaphognathite (modified second maxilla) in the exhalent passage (McMahon and Wilkens, 1983).

The blue crab circulatory system, like that of other crustaceans, is an open system. However, the hemolymph is contained in vessels throughout much of the body. The force necessary for hemolymph circulation is provided by the single-chambered heart. Hemolymph in the pericardial sinus enters the heart through openings called ostia and exits through the posterior and anterior aortae. Numerous arteries carry the hemolymph from the aortae to the various tissues and organs. There are no closed venous vessels. After passing through the smaller vessels of the

arterial system, hemolymph enters the hemocoel and passes through discrete channels before collecting in the venous infrabranchial sinus (McLaughlin, 1982). From here hemolymph passes through the limb bases and the afferent branchial channels to perfuse the gill filaments. The flow of the hemolymph in the gill lamellae is countercurrent to the flow of water in the branchial chamber (Hughes, Knights and Scammel, 1969). After leaving the gills via the efferent branchial channels the hemolymph moves through the branchiopericardial "veins" to reach the pericardial cavity (McMahon and Wilkens, 1982).

A comprehensive list of respiratory and circulatory parameters in the blue crab was recently summarized by Cameron (1986). On a per gram body weight basis, gill surface area in C. sapidus ($710\ mm^2\ g^{-1}$) is much larger than that of most brachyuran crabs (e. g. Uca, Sesarma, Ocypode, etc. from Gray,1957). This probably reflects the active swimming lifestyle of the Portunidae. The oxygen consumption rate ($\dot{M}O_2 = 85.6\ ml\ kg^{-1}\ hr^{-1}$) is correspondingly greater than for other crab species. The ventilation rate ($\dot{V}_W$) of 111 ml min^{-1} reflects a respiratory frequency of 31.7 min^{-1} given a combined gill chamber volume of 3.5 ml. The unusually low cost of ventilation (0.02% of $\dot{M}O_2$) suggests that the water pumping mechanism is highly efficient.

An unexplained aspect of decapod respiration is the phenomenon of current reversal in the gill chamber. The scaphognathite pump reverses the direction of its beating, causing

water to enter the normally exhalent channel, cross the gills and exit via the Milne-Edwards openings. This may occur 0-50 times per hour in Callinectes sapidus (Batterton and Cameron, 1978) for brief periods of around 5 seconds (Hughes et al., 1969). Numerous functions have been suggested for these reversals, such as enhanced ventilation of normally poorly ventilated regions of the gills (Arudpragasam and Naylor, 1964), removal of detritus from the gills (Hughes et al., 1969) and cleaning of intake filters (McMahon and Wilkens, 1983). Adequate experimental evidence has not been presented for a general explantation for the phenomenon. Frequency of reversals did increase when crabs were exposed to increased salinity or CO_2 (Batterton and Cameron, 1978) or to air (Taylor, Butler and Sherlock, 1973).

Other characteristic features of crustacean respiration are intermittent cardiac and ventilatory pauses. Crustaceans at rest in well-oxygenated, undisturbed environments often discontinue ventilation and heart beat for periods of up to several minutes (McMahon and Wilkens, 1977). This may be a way of conserving energy when hemolymph oxygen levels are near maximum (Batterton and Cameron, 1978).

Although an open circulatory system connotes sluggish movement of hemolymph through open spaces at low velocity and low pressure, this is not true for the decapods, particularly active ones such as blue crabs. Resting heart rate (f_H= 89 beats min^{-1}) and cardiac output ($\dot{V}_B$ = 151 ml kg^{-1} min^{-1}) for the blue crab can increase dramatically during exercise. Cardiac stroke volume

(Sv_C), 1.70 ml $kg^{-1}beat^{-1}$ at rest, also increases during exercise (Booth, McMahon and Pinder, 1982). Although blood flow velocity has not been measured in blue crabs, other decapods have arterial flow velocities approaching 10 cm sec^{-1}, and ventricular systolic pressures of 10-36 Torr (1.3 - 4.8 kPa) and diastolic pressures of 4-16 Torr (0.5 - 2.1 kPa; McMahon and Wilkens, 1983). These characteristics along with the large hemolymph volume (approximately 30% wet weight; Gleeson and Zubikoff, 1977) indicate an efficiency comparable to closed circulatory systems of other aquatic animals of similar activity level (McMahon and Wilkens, 1983).

Animals have been experimentally manipulated to reduce the supply of respiratory gases or increase the demand to evaluate the capabilities of the gas exchange system. The responses of the blue crab to hypoxia (low O_2) and hypercapnia (high CO_2; Batterton and Cameron, 1978; Cameron, 1985), as well as exercise (Booth et al., 1982; Booth, McMahon, deFur and Wilkes, 1984; Milligan et al., 1989) has been previously examined. In each case blue crabs make ventilatory and/or circulatory adjustments to maintain O_2 delivery and CO_2 elimination to and from metabolizing tissues. Hemolymph pH and lactate levels may change, but because these allosteric modulators have opposing effects on hemocyanin-O_2 affinity, O_2 delivery and CO_2 elimination are not compromised (Milligan et al., 1989). This is an example of enantiostatic regulation (Mangum and Towle, 1977).

Octolasmis muelleri

Octolasmis is a genus of gooseneck (or stalked) barnacle (Cirripedia:Thoracica). They attach to the gills of decapod crustaceans as ectocommensals (Newman, 1967). Octolasmis muelleri (considered synonymous with O. lowei by Nilsson-Cantell 1927) adult stages have been found in the gill chamber of numerous brachyuran crabs (Humes, 1941; Walker, 1974). After internal fertilization and brooding by the parent (Jeffries and Voris, 1983), the barnacle goes through six naupliar stages and one cyprid stage as a free-swimming planktivore, before settling down on a crab host (Lang, 1976). The adult barnacle, cemented to the gill lamellae of the host crab, filter feeds on particulate matter in the ventilatory stream. The barnacle derives no nutrients directly from the crab and is thought to harm its host only indirectly by occluding the ventilatory current when infestation levels are great (Walker, 1974). Therefore the Octolasmis-Callinectes relationship is generally considered to be a commensalism, in which the barnacle is benefitted and the crab is unaffected. This belief will be evaluated in the following chapters.

The barnacle should experience selection pressure to select optimal (i. e., better ventilated) sites within the host branchial chamber. In addition the barnacle should select optimal (non-molting adults) hosts because, during the host molt, it would be

shed with the exoskeleton covering the gills and is not thought capable of survival outside the host (Walker, 1974). The possibility of Octolasmis muelleri host and site selection will be evaluated in Chapter 1. Because the barnacle is dependent on the host for the continued renewal of ventilatory water, selection pressure on the barnacle should minimize any detrimental impact on the host. This will be discussed in Chapters 2 and 3.

Host:Gill-Symbiont Relationships

The distribution of ectoparasites on gills has been studied in several species of fish (Walkey, Lewis and Dartnall, 1970; Suydam, 1971; Hanek and Fernando, 1978; van den Broek, 1979; Davey, 1980). Frequently, nonrandom distributions have been discovered, but the cause of these distributions has not been satisfactorily demonstrated. Site specificity for the best ventilated parts of the gill chamber has been attributed to passive dispersal of parasite larvae in the ventilatory stream (Walkey, Lewis and Dartnall, 1970; Suydam, 1971; van den Broek, 1979). In fact, Paling (1968) assumed that parasitic glochidia were distributed passively by the ventilatory stream of the brown trout Salmo trutta. He used the parasite distribution to estimate the volumes of water passing over the parts of the gill chamber. His assumptions were validated by more sophisticated experiments using marker dyes (Hughes and Morgan, 1973). Davey (1980) attributed the distribution of male copepods, Lernanthropus kroyeri, on bass gills to passive

distribution, but was unable to explain satisfactorily the preference of females for the internal hemibranchs of the gills without invoking active site selection

The effects of endoparasites on fish respiration have been examined (Lester, 1971; Giles, 1987), but the effects of branchial ectosymbionts are unstudied. Even less information is available for crustaceans and their branchial symbionts. The isopod, Probopyrus pandalicola, causes decreased oxygen consumption in its intermediate host, Acartia tonsa (Anderson, 1975a), and in most cases in its definitive host, Palaemonetes pugio (Anderson, 1975b). In the former, the ectoparasitic isopod may reach a greater mass than the host. In the latter, the isopod has an endoparasitic stage and an ectoparasitic stage that increases in size until it takes up the entire host branchial chamber (Anderson, 1990). In both cases the parasite feeds on host hemolymph.

The O. muelleri-C. sapidus system differs in several important ways. This ectocommensal is much smaller than the host and does not feed on host hemolymph. This allows measurement of the effect of ectocommensal presence without the added effects of host hemolymph loss. In this system the host's compensatory response to a natural obstruction in the ventilatory stream can be evaluated.

The results of this investigation will be important on three levels: organism, population and general host/commensal relationship. On the organismal level it will add to our understanding of the gas exchange system in the blue crab. On the

population level it will provide preliminary information on the impact of a potentially harmful commensal organism on a commercially important crab species. Finally, the results of this study should shed some light on the interaction and possible coevolution between host/commensal physiological systems.

CHAPTER 1
DISTRIBUTION OF Octolasmis muelleri WITHIN THE HOST GILL CHAMBER AND IN THE BLUE CRAB POPULATION OF CEDAR KEY, FLORIDA

Introduction

Adult Octolasmis muelleri live in gill chambers of decapod crabs, generally attached to the gill lamellae (Humes, 1941). The adults produce many broods of larvae during warmer months (Jeffries and Voris, 1983). The larval phase consists of six naupliar stages that feed on phytoplankton and one nonfeeding cyprid (Lang, 1976). Under laboratory conditions (24-29° C) the nauplius to cyprid transition takes 14-18 days (Lang, 1976). The cyprid stage enters the branchial chamber of a crab on the inhalent respiratory current and cements its antennules to two adjacent gill lamellae (Walker, 1974). Metamorphosis to the adult stage then takes place over the next 20-72 hours (Lang, 1976). Sexual maturity is reached in the tropical species Octolasmis cor within two weeks after metamorphosis (Jeffries, Voris, and Yang, 1985); development may not be as rapid in O. muelleri in temperate areas.

Because the adult barnacle cannot move once attachment is complete, selection of the appropriate site within the host gill

chamber is critical to the barnacles survival and reproduction. Because the barnacle is attached to the host exoskeleton, it is shed when the host molts and ultimately dies (Walker, 1974). Selection of a host with an intermolt period long enough to allow barnacle maturation and reproduction is therefore essential. This study was undertaken to determine the characteristics of natural Q. muelleri infestations in blue crabs with a view to assessing host and site selection by the barnacle. In addition, infestation levels in the blue crab population of the Cedar Key area were evaluated to examine the possibility that the barnacle has a detrimental impact on the host population.

The settling cyprid larvae of some symbiotic cirripedes are thought to respond to host-related chemicals and host breeding cycles, as well as the stimuli to which free living barnacle cyprids respond (i.e., chemicals, currents, textures, light, gravity, and hydrostatic pressure) (Lewis, 1978). The host specificity exhibited by some of the 10 Octolasmis species found on decapods in the seas around Singapore (Jeffries, Voris and Yang, 1982) suggests that the larvae exercise some discrimination in host selection. Jeffries, Voris and Yang (1989) have found evidence that cyprid larvae of Q. cor and Q. angulata aggregate on premolt crabs (Scylla serrata) and transfer to the newly molted crab for attachment, indicating fine discrimination of host status and delayed metamorphosis. Cyprid larvae survived up to 177 days, although their competence for metamorphosis at that time is unknown (Jeffries et al., 1989). Similar capabilities in the closely related Q. muelleri would allow

selection of optimal hosts and attachment sites within hosts. One expects that the ideal host is a large adult crab already infested with O. muelleri. Such a host would molt infrequently, provide greater settlement area, be least harmed by the presence of the barnacle, and contain mating partners. Adult male blue crabs molt two to three times after reaching maturity (Van Engel, 1958). Females may molt after reaching maturity (Havens and McConaugha, 1990). One might expect settling barnacles to favor the hypobranchial side of the gills where water enters the branchial chamber, particularly in the region of gills 3, 4, 5 and 6, which are the best ventilated in the crab Carcinus maenas (Hughes et al., 1969) and presumably also in the blue crab. The hypotheses of optimal site and host selection will be compared to the null hypothesis of random settlement.

Whether the host can deter barnacle larvae from settling, or influence subsequent survival is unknown. For example, the grooming action of the host epipodites may affect barnacle survival, especially during and immediately after settlement. Otherwise molting would appear to be the only host defense against Octolasmis (Walker, 1974).

Methods

Blue crabs were trapped monthly from June 1987 to May 1988 at Seahorse Key on the Gulf coast of Florida (29° 06' N lat., 83° 04' W long.), using plastic coated wire mesh commercial crab traps.

Trapping effort was not equal for each month. Captured crabs were sexed and their width measured. Because lateral horns were frequently broken, short width (the distance between the notches just anterior to the lateral horns) was used as the size measurement. Although short width is less commonly used than full width, it more accurately represents the blue crabs' size (Olmi and Bishop, 1983). Crabs were visually inspected for ectocommensal carapace barnacles (Chelonibia patula) and gill barnacles (O. muelleri). Attachment sites were recorded with respect to gill chamber (right or left), aspect (epibranchial or hypobranchial), gill number (#1 - 8, anterior to posterior), and distance along the gill. For the latter measurement, each gill was arbitrarily divided into thirds (basal, medial, and distal), which, because of the triangular nature of the phyllobranch gill, made up 56%, 33% and 11%, respectively, of the gill surface area. Because the gills are tightly oppressed, the only area available to settling barnacle larvae is the edges of the gill lamellae facing into the ventilatory stream, which shall be referred to as the "leading gill surface area." I measured the gills of four blue crabs with calipers and the averaged the results to determine the proportion of the total leading gill surface area made up by each gill.

The commercial traps almost exclusively captured adults. A seagrass flat 100 m north of the trapping site was dredged in April 1988 and 105 juvenile crabs were captured. This gave the size distribution of infested crabs from the juvenile stages up to the

adult stages. These crabs were evaluated in the same way as were the trapped adult crabs.

Results

Infestation Summary

The monthly crab catches are given in Table 1-1. Because trapping effort was not equal in each month, the monthly sample sizes are not comparable as population estimates. The trapping appeared heavily biased towards adult male crabs, which made up 81% of the total catch. Catch sizes, sex ratios and infestation rates fluctuated from month to month with few apparent seasonal trends. In all months male crabs had a greater or equal infestation rate than females. The number of *O. muelleri* per infested crab reached a peak in the spring with the April rate an order of magnitude greater than other months and the mean (21.7 *O. muelleri* per infested crab). The mean number of *O. muelleri* per crab was inflated by a small number of heavily infested crabs. The overall median infestation level was 4 barnacles per crab. No obvious age classes of infesting barnacles existed. Heavily infested crabs contained barnacles of many different sizes, ranging from freshly metamorphosed adults (about 1 mm total body length) to large mature adults (up to 2 cm total body length).

Distribution Among Hosts

The number of barnacles per infested crab host can be seen in Figure 1-1 as the frequency distribution of O. muelleri on crabs. The 318 uninfested crabs are not included in the figure. The 211 infested crabs contained from 1 to 432 barnacles. This distribution is not significantly different from the negative binomial distribution ($P < 0.01$). When the left and right gill chambers of the host crab are treated as separate entities, the frequency distribution is similar (fig. 1-2) and again not different from the negative binomial ($P < 0.01$).

For most sample months no difference existed between the mean carapace short width of infested and uninfested crabs (t-test, $P > 0.05$). However, the sampled crabs were almost exclusively adults. Figure 1-3 contains the size distribution of the infested and uninfested crabs from the April 1988 sample, which contained 61% juveniles. The mean short width of infested crabs was 11.6 cm, that of uninfested crabs was 5.3 cm. These means were compared with a t-test and are significantly different ($P < 0.001$). None of the juvenile crabs were infested.

The presence of another ectocommensal barnacle, Chelonibia patula, was observed on a significant number of our sample crabs (21.5%). The co-occurrence of C. patula and O. muelleri is reported in a contingency table (table 1-2). The Pearson chi-square test for independence (Feinberg, 1981) of the two infestations shows that they are not independent ($P < 0.001$). Crabs that contain one of

these two ectocommensal barnacles are more likely to harbor the second than crabs not infested with the first.

Distribution Within the Host

Inside the host gill chamber Octolasmis muelleri were found attached to either side of each of the gills, the hypobranchial gill chamber wall, the epibranchial gill chamber membrane, the scaphognathite, and the hypobranchial and epibranchial gill rakers. The majority of barnacles were found on the hypobranchial side of the gills (93%), but the epibranchial side was infested only when the hypobranchial side was heavily infested.

Although the distribution of barnacles along the gill filament favored the basal third (54%), the proportion of barnacles found on each third of the gill length (medial - 33%, distal - 10%) was equivalent (to the nearest 1%) to the leading surface area made up by that region of the gill. However, the proportion of O. muelleri found on each of the eight gill filaments was not equivalent to the proportional leading surface area of that gill. This comparison is shown in Table 1-3. Using the proportion of total leading gill surface area as the expected proportional share of barnacles for each gill, a chi-square test indicates that the barnacle distribution is significantly different from expected (X^2 = 155, 7df; $P < 0.001$). Fewer barnacles than expected were found on gills 1, 7 and 8. Gills 3, 4 and 6 were more heavily infested than expected.

Discussion

The infestation rate of Octolasmis muelleri in the blue crabs of Seahorse Key (40%) is not different from that reported for blue crabs at Grand Isle, Louisiana (37%) by Humes (1941). Curiously, in the present study, 45% of the males and 20% of the females were infested, yet Humes (1941) found 19% of the males and 43% of the females infested. This rate and the number of barnacles per infested crab (21.8) indicate a very prevalent symbiont. If these barnacles have a negative effect on the host, their presence could have a major impact on the C. sapidus population. The physiological effect of infestation on the host is explored in Chapters 2 and 3.

The hypothesis that O. muelleri larvae would select optimal hosts was first tested by comparing the barnacle distribution to the negative binomial distribution, a fundamental model frequently used to describe host-parasite distributions (Williams, C. B.,1964). It is used here to model the frequency distribution of parasites on hosts, and the distribution of parasites on host gill chambers. The negative binomial distribution is theoretically applicable to this system because a random distribution is not expected due to variation in host characteristics (age, size, sex, and habitat), that makes the chance of infestation unequal (Crofton, 1971). The close fit to the negative binomial and the low k values (k = .14 and .13 for barnacles/crab and barnacles/gill

chamber, respectively) indicate extreme aggregation (Williams, C. B., 1964). Barnacles are most likely to be found on previously infested gill chambers and hosts. This may be due to barnacle larvae being stimulated to settle by the presence of conspecifics as has been shown in other barnacle species (Crisp, 1974) or to certain hosts being more attractive or having greater exposure to barnacle larvae. The low and similar k values for the host distribution and gill chamber distribution suggest that both effects are important. It is likely these effects enhance each other. It is improbable that Q. muelleri larvae are reinfesting their parents host, since they must undergo a 2-3 week development period as plankton (Lang, 1976).

A truncated form of the negative binomial distribution has been successfully used to model distributions in which the parasite incurs mortality in heavily infested hosts (Lanciani and Boyett, 1980). Hosts with large parasite loads would die and therefore not be sampled, causing underrepresentation in the larger parasite load classes. The Q. muelleri distribution on C. sapidus exhibits overrepresentation in the larger parasite load classes . Although not conclusive, this suggests that either Q. muelleri infestation does not affect C. sapidus mortality or that the effect is linear (Lanciani and Boyett, 1980).

When samples of adult crabs were compared, no difference existed between the size (carapace short width) of infested crabs and uninfested crabs. A large difference between the size of infested and uninfested crabs was evident only when immature

crabs were considered. Immature crabs are unsuitable hosts because of their short intermolt period which is less than one month in the juveniles up to stage 14 (Millikin and Williams, 1984). Barnacles infesting immature crabs may escape detection due to small body size (Walker, 1974). However, in this study careful inspection of 105 immature crabs did not reveal any that harbored O. muelleri. Infested immature crabs have been observed only occasionally since this study was completed (unpublished observations).

Although the ectocommensal carapace barnacle Chelonibia patula does not share the microhabitat of O. muelleri, one might predict that certain crabs are ideal hosts for both barnacles based on distribution criteria such as infrequency of host molting, greater settlement area and host resistance to harmful effects. The evidence that the two barnacle species tend to select the same hosts supports this view: however, it is equally possible that infestation by one barnacle species weakens the crab and makes it more susceptible to infestation by the other. It is also likely that the longer the duration since the last crab molt, the more time it has had to accumulate ectocommensal barnacles of either species. Less likely is the possibility that the planktonic larvae of the two barnacle species complete development and settle in the same localities, infesting crabs there.

Within crab gill chambers, adult O. muelleri were found attached to virtually every surface in heavily infested crabs. This negates the hypothesis of settlement and survival only on

optimally ventilated sites. Infestations of less than 10 Octolasmis were usually confined to the actual gill tissue on the hypobranchial side. Concentration of the barnacles on the middle gills agrees with the distribution found for the Beaufort, North Carolina population by Walker (1974) and Jeffries and Voris (1983). The basal and medial portions of the hypobranchial side of gills, 3, 4, 5 and 6, which could be considered the optimal site for barnacle growth, contained 61.4% of the barnacles sampled. This area constitutes only 28.6% of the available gill surface area, and an even smaller proportion of the total surface area used for attachment (about 5.7%). The observed aggregation of barnacles could be explained either as site selection by gregarious settling cyprid larvae, or simply the result of passive distribution in the mainstream of the crabs ventilatory current.

In summary, aggregation of Octolasmis on the expected optimal hosts and at the expected optimal sites within the gill chamber have been demonstrated. This suggests host and site-within-host selection by barnacle larvae. However, alternate hypotheses have been advanced to explain the observed distribution and warrant further investigation.

Table 1-1. Octolasmis muelleri/Callinectes sapidus population infestation summary 1987-1988.

	Crab population			Infested crabs			
Month	Total	Male	Female	% of Total	%Male	%Female	Number of Octolasmis/ infested crab
May	52	44	8	29	100	0	6.6
June	41	37	4	59	100	0	6.9
July	91	73	18	17	80	20	13.9
Sept	35	28	7	29	100	0	16.2
Oct	100	84	16	67	94	6	8.5
Nov	58	47	11	45	92	8	13.4
Dec	29	27	2	45	100	0	11.0
Feb	13	4	9	62	38	63	45.5
Mar	40	22	18	33	54	46	15.7
Apr	70	63	7	29	100	0	116.4
Total	529	429	100	40	91	9	21.1

Table 1-2. Contingency table for infestation of crabs by Chelonibia patula and/or Octolasmis muelleri, (values are for observed number of crabs/expected number of crabs). Significantly more crabs are observed with both barnacle infestations or neither infestation than expected ($p < 0.001$).

		Octolasmis muelleri	
		+	-
Chelonibia patula	+	63/31.5	19/50.4
	-	84/115.4	216/184.6

Table 1-3. Comparison of Octolasmis muelleri distribution with facing gill surface area.

Gill number	Proportion of Total Gill Area	Expected number of barnacles	Observed number of barnacles	Difference
1	0.016	25	8	-17
2	0.025	38	45	+7
3	0.092	141	231	+90
4	0.162	249	273	+24
5	0.210	323	330	+7
6	0.186	286	324	+38
7	0.169	260	237	-23
8	0.141	217	89	-128

Figure 1-1. Frequency distribution of Octolasmis on blue crabs. Individual crabs with 281, 298, 362, and 432 Octolasmis are not shown in the figure. Combined data from 529 crabs.

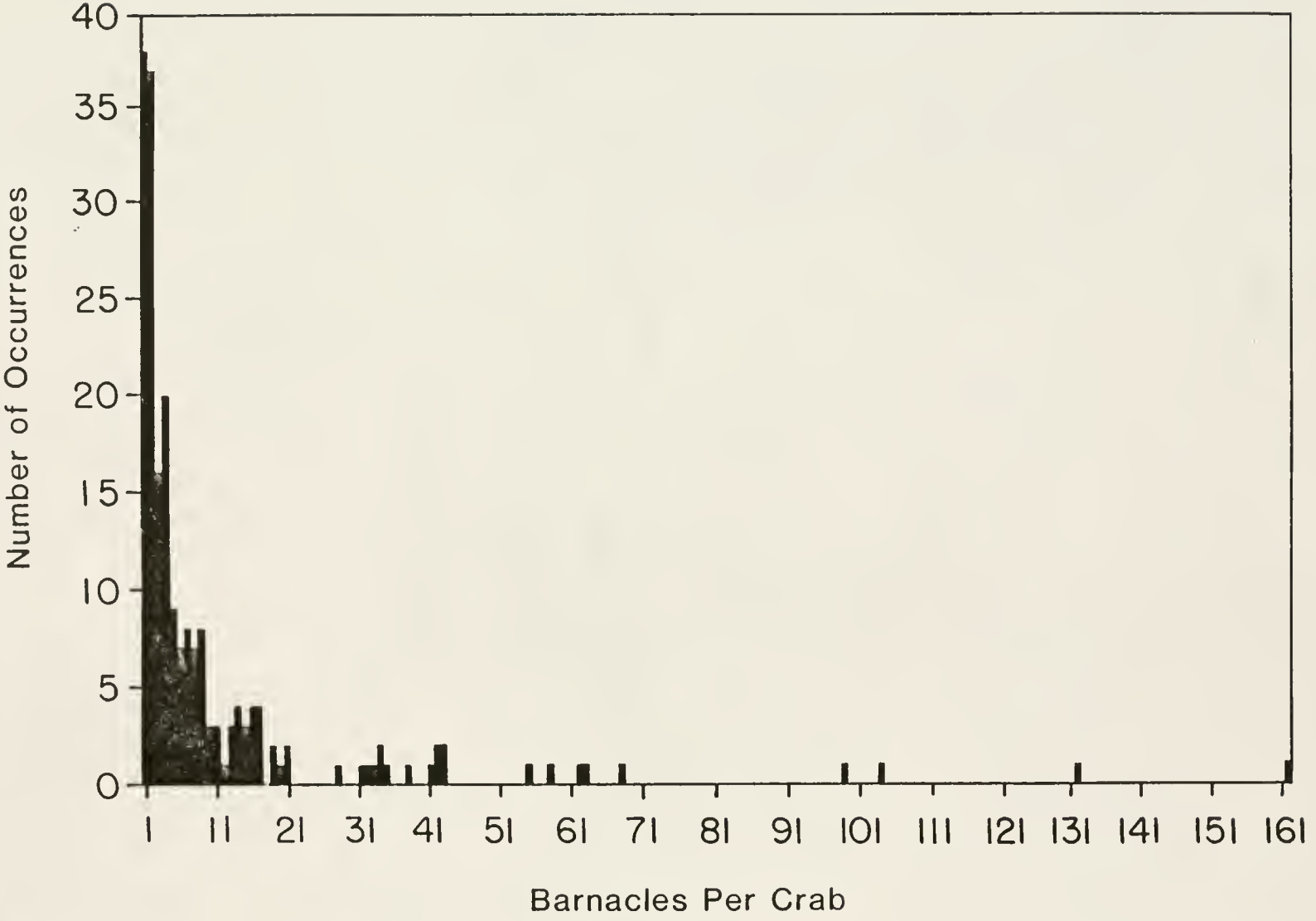

Number of Occurrences
40
35
30
25
20
15
10
5
0
1
11
21
31
41
51
61
71
81
91
101
111
121
131
141
151
161
Barnacles Per Crab

Figure 1-2. Frequency distribution of Octolasmis on blue crab gill chambers. Individual gill chambers with 208 and 224 Octolasmis are not shown in the figure. Combined data from 529 crabs.

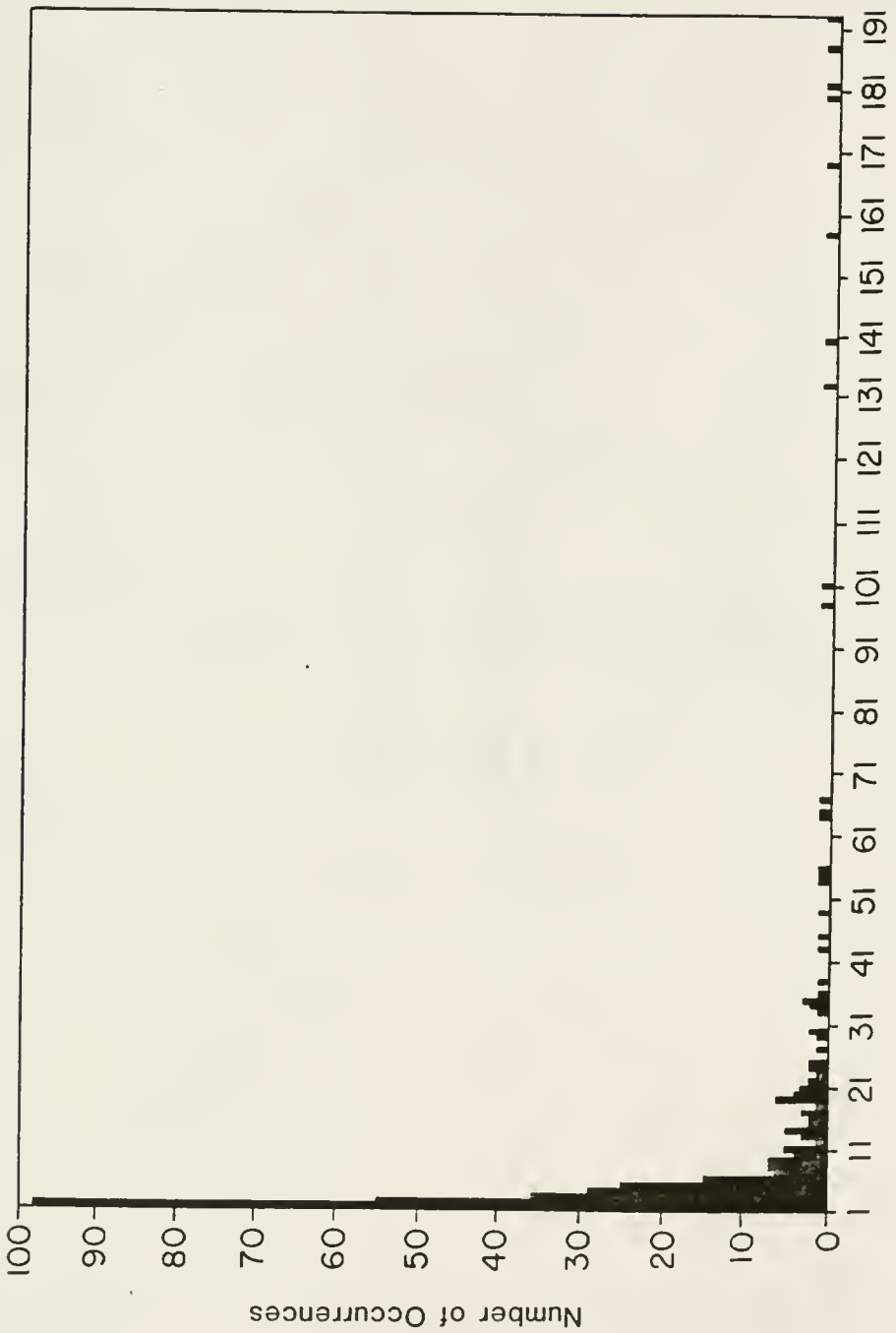
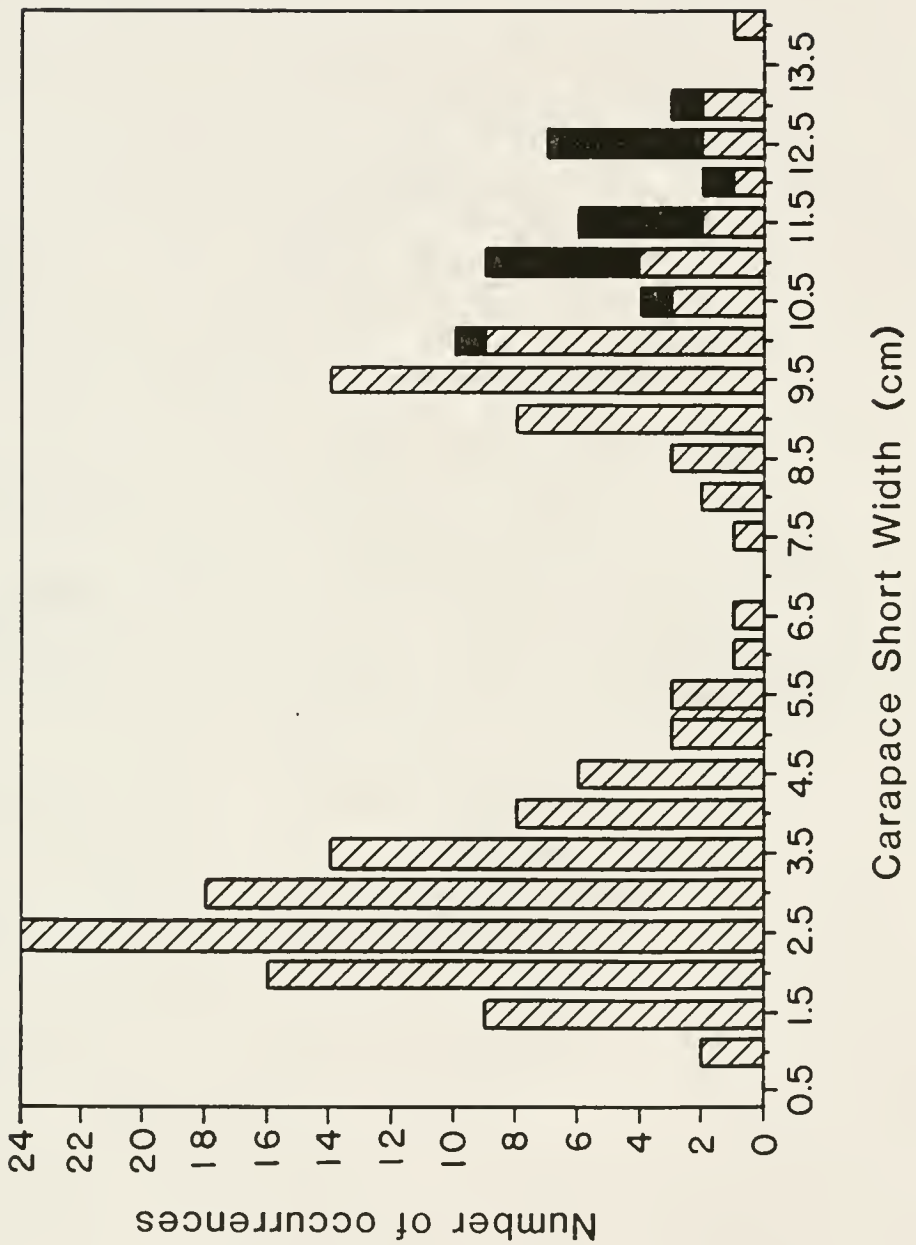

Number of Occurrences
100
90
80
70
60
50
40
30
20
10
0
1
11
21
31
41
51
61
71
81
91
101
111
121
131
141
151
161
171
181
191
Barnacles Per Gill Chamber

Figure 1-3. Size distribution of Octolasmis muelleri-infested and non-infested blue crabs. Sizes are given as carapace short widths in cm. Darkened bars are infested crabs. Cross-hatched bars are noninfested crabs.


Number of occurrences
0
2
4
6
8
10
12
14
16
18
20
22
24
0.5
1.5
2.5
3.5
4.5
5.5
6.5
7.5
8.5
9.5
10.5
11.5
12.5
13.5
Carapace Short Width (cm)

CHAPTER 2
EFFECTS OF Octolasmis muelleri INFESTATION ON BLUE CRABS

Introduction

The respiratory physiology of aquatic decapod crustaceans has been well-studied (see reviews by Taylor, 1982; McMahon and Wilkens, 1983; Mangum, 1983; Truchot, 1983; Cameron, 1986). Experimental methodology has involved changes in the external respiratory medium such as hyperoxia (Dejours and Beekenkamp, 1977; Sinha and Dejours, 1980; Wheatly, 1987), hypoxia (McMahon, Burggeren and Wilkens, 1974; Butler, Taylor and McMahon, 1978; Wheatly and Taylor, 1981) and hypercapnia (Henry, Kormanik and Cameron, 1981; Cameron, 1985). Respiration of the blue crab, Callinectes sapidus, has been particularly well studied with respect to the above environmental stressors as well as molting (Cameron and Wood, 1985), emersion (Batterton and Cameron, 1978) and exercise (Booth et al., 1982; Booth et al., 1984). The wide distribution of this species from fresh water to hypersaline lagoons and temperate regions to the tropics (Williams, 1984), confirms what physiological studies suggest, namely an ability to tolerate a wide range of environmental conditions.

Many gill breathers are hosts to parasites and ectocommensals that could affect respiration. Endoparasitic cestode larvae (Schistocephalus) cause increased oxygen consumption in stickleback fish, Gasterosteus aculeatus (Lester, 1971), and stimulate the host to breath more often at the surface in hypoxic waters (Giles, 1987). Snails infested with larval trematodes have higher metabolic rates than do uninfested controls (Duerr, 1967; Vernberg and Vernberg, 1967). Crustacean endoparasites can also have profound effects on host metabolism. Blue crabs are hosts to rhizocephalan barnacles such as Loxothylacus texanus. These endoparasitic barnacles stunt host growth (Overstreet, 1982) and stimulate male hosts to develop female secondary sex characteristics (Reinhard, 1956). Baffoni (1953) however, was unable to show any effect of rhizocephalan infestation on host metabolic rate.

In all of these examples the parasites feed on host tissue and make up a significant part of the host weight. They also kill or severely debilitate the host. The direct effects of parasite presence on the host are not separable from the effects of blood/hemolymph depletion, increase in biomass, or parasite associated disease. In addition, in the first two systems, the parasite larvae induce host behavior that make the host more susceptible to predation by the parasites' definitive host (Lester, 1971; Giles, 1987).

The effect of gill parasites on host respiration has been measured only for the isopod Probopyrus pandalicola. Decreased

oxygen consumption occurs in both its intermediate host, the copepod Acartia tonsa (Anderson, 1975a), and in most cases, its definitive host, the shrimp Palaemonetes pugio (Anderson, 1975b). In the former host the isopod is ectoparasitic and not a gill parasite. In the latter the female isopod has both an endoparasitic phase of 1-2 weeks (Anderson, 1990) and a branchial phase in which it feeds on host hemolymph (Anderson, 1975b). The male is much smaller and associates with the female in the host branchial chamber (Anderson, 1990). Significant host mortality occurs in the early periods of infection, but not when the parasite is in the branchial stage (Anderson, 1990). This is surprising, given that the parasite grows to such a size that it distorts the shape of the host branchial chamber (Anderson, 1975b). The decrease in metabolic rate of parasitized shrimp is attributed to changes in host lipid metabolism (Anderson, 1975b). This may be related to the effect of the parasite on the host reproductive system - "parasitic castration" -inhibition of ovarian maturation and disruption of development of male secondary sexual characteristics (Beck, 1980). Thus, the effects of the parasite on gas exchange per se are not separable from the effects caused by feeding on host hemolymph.

The Octolasmis muelleri - Callinectes sapidus system offers a unique opportunity to examine the effects of a branchial commensal on host gas exchange including determination of a possible detrimental effect on the host as well as evaluation of the host mechanisms of compensation.

The aim of this study was to measure the effects of O. muelleri infestation on the following parameters of blue crab respiration: oxygen consumption, heart rate, ventilation rate, and the hemolymph oxygen tension, oxygen content, carbon dioxide content, pH, and lactate concentration.

Methods

Animal Maintenance

Adult blue crabs (150 - 250 g) were collected in commercial traps at Seahorse Key on the Gulf coast of Florida (29°06′ N. lat., 83° 04′ W long.) from April 28, 88 to September 4, 1988. Less than 30 crabs were maintained at any one time in an 800 l tank of recirculating filtered seawater (33-36 ppt salinity) at 23-26°C on a 12 hr light:12 hr dark cycle at the University of Florida in Gainesville. The crabs were fed chopped fish 1-2 times weekly, but food was withheld 48 hours prior to experimentation. Intermolt crabs (stage C-4, Johnson, 1980) were given a minimum of one week to acclimate to these holding conditions before use in experiments.

Experimental Protocol

Measurements were made on three series of experimental blue crabs. Each series contained crabs that were naturally infested by the barnacle, Octolasmis muelleri, and uninfested control crabs. This was a blind experiment, infestation level could

not be determined until the crab was killed. Series A crabs (n = 30) were used to measure whole-animal respiratory parameters (oxygen consumption rate, $\dot{M}O_2$; heart rate, f_H; and ventilation rate, f_{SC}). Series B crabs (n = 40) were used to measure prebranchial (venous,v) hemolymph characteristics (pH; oxygen tension, PO_2; total carbon dioxide content, C_{CO_2}; and (L+)-lactate concentration). Series C crabs (n = 22) were used to determine both prebranchial and postbranchial (arterial,a) hemolymph characteristics (pH, PO_2, oxygen content (C_{O_2}) and (L+)-lactate concentration). After measurements were completed, crabs were killed by freezing and the gill chambers were opened and visually inspected for the presence of ectocommensal barnacles (Gannon, 1990; Chapter 1). The total number of barnacles present in the gill chamber of each crab was used as an index of infestation level.

Analytical Techniques

Series A crabs were prepared for heart and ventilation rate recording and placed in a continuous flow respirometer (Taylor, Butler and Al-Wassia, 1977). Depending on body size, crabs were placed in either a 1.7 l or 3.8 l cylindrical respirometer that was placed in a 38 l water bath. Filtered seawater (22-25°C, 33-35 ppt salinity) was pumped through the respirometer at a constant rate that ranged from 60 to 95 ml min^{-1}. Sampling ports in the inflow (incurrent) and outflow (excurrent) of the respirometer allowed simultaneous withdrawal of 1 ml water samples for measurement

of water PO_2. The PO_2 was determined using an IL O_2 electrode (20984) thermostatted to 25°C and connected to an IL 213 blood gas analyzer. The $\dot{M}O_2$ was calculated in μmol kg^{-1} min^{-1} using the following equation:

$$\dot{M}O_2 = \frac{\Delta PO_2 \ \alpha O_2 \ f}{m}$$

where ΔPO_2 is the difference between incurrent and excurrent PO_2 (in kPa), αO_2 is the solubility coefficient (in μmol O_2 ml water $^{-1}$ kPa^{-1}; Jones, 1972), f is the flow rate through the respirometer (in ml H_2O min^{-1}), and m is body mass in kg.

The f_H and f_{SC} in both branchial chambers were measured continuously with an impedance conversion technique (Dyer and Uglow, 1977). In the case of heart rate, two holes were drilled through the carapace but not penetrating the epidermis on either side of the heart and the end of a lacquered copper wire (32 gauge) stripped for 3 mm was inserted into each hole through the epidermis, to a depth of 4 mm. They were held in place with cyanoacrylate glue and latex dental dam. These wires led outside the respirometer to an impedance converter (UFI 2991) and the output was displayed on a Grass model 79 polygraph. The f_{SC} was recorded similarly, inserting the recording wire into the exhalent canal of each branchial chamber and the reference wire onto the carapace. Crabs were allowed at least 48 hours to recover from the

surgical procedures and to acclimate to the respirometer before measurements were taken.

Prebranchial hemolymph samples (l ml) of series B and series C crabs were removed from the infrabranchial sinus by penetrating the arthrodial membrane at the base of the swimming leg with a 22 gauge needle attached to a chilled syringe. Crabs in series C were prepared for postbranchial hemolymph sampling by drilling a hole directly over the heart, through the carapace but not penetrating the epidermis. The hole was sealed with a l cm^2 patch of latex dental dam and cyanoacrylate glue. The postbranchial hemolymph sample was withdrawn by puncturing the dental dam patch with a 22 gauge needle attached to a chilled syringe. These crabs were give 48 hours to recover from this surgery before experimentation.

Crabs in series B and C were placed individually in 12 l of aerated filtered seawater (22-25°C and 33-35 ppt salinity) in a 20 l covered container and allowed at least 2 hours to acclimate before pre- and postbranchial hemolymph samples were taken as outlined above. For each hemolymph sample from a series B crab, the PO_2 of an 880 µl subsample was measured as outlined above for water samples. The pH of the same subsample was also measured in the IL 213 blood gas analyzer using an IL pH electrode (20982). The (L+)-lactate concentration was measured on an 80 µl hemolymph subsample using a commercial reagent kit (Sigma #826). The C_{CO_2} of a 40 µl subsample was measured with a Capnicon automatic

analyzer (Cameron Instruments Company). For each hemolymph sample from a series C crab, the PO_2 and pH of a 920 µl subsample were measured as described above. The C_{O2} of an 80 µl subsample was measured with a Cavitron LexO_2con oxygen analyzer.

Statistical Analysis

Values measured for series A and C infested crabs were compared with those recorded for uninfested crabs using Student`s t-test for independent means (Sokal and Rohlf, 1969). Values measured for series B uninfested, moderately infested, and heavily infested crabs were compared using a Model I single classification ANOVA (Sokal and Rohlf, 1969). Wherever the variances were not found to be homogeneous with the F-test or the Fmax-test, a t-test for samples with unequal variance was used (Sokal and Rohlf, 1969). Differences were considered significant at $P < 0.05$.

Results

Experimental crabs were all of similar size (mean mass = 195.7 ± 37.2 g S. D.). This minimized effects of mass on $\dot{M}O_2$, f_H and f_{SC}. These values were each plotted against mass on a log:log scale but no linear relationships were found. This suggests that the influence of mass was negligible over this narrow range. Therefore the measured values for f_H and f_{SC} were not adjusted for mass. To facilitate comparison with other species, $\dot{M}O_2$ was reported per unit mass.

Oxygen consumption rates of infested series A crabs were not significantly different from those of uninfested crabs (Table 2-1); however the infested crabs had elevated f_H (44%) and f_{SC} (83%).

For the comparisons of prebranchial hemolymph parameters, series B crabs were divided into three classes based upon intensity of infestation as follows: uninfested, which includes lightly infested crabs (from 0 to 6 barnacles), moderately infested (10-20 barnacles) and heavily infested (more than 20 barnacles). These divisions were based on an analysis of the distribution of Q. muelleri on 512 blue crabs (see Chapter 1; Gannon, 1990). Four heavily infested crabs (each containing more than 50 barnacles) did not survive handling and surgical preparation.

The mean prebranchial hemolymph pH, PO_2, C_{CO_2} and lactate concentrations of the heavily infested crabs were not significantly different from those of the uninfested crabs (Table 2-2); however moderately infested crabs exhibited lower hemolymph pH (0.3 pH units) and C_{CO_2} (25% reduction) and almost double the PO_2 of control crabs. Lactate concentrations were virtually identical in all groups; however the small sample size in the moderately infested category precludes statistical comparison.

Pre- and postbranchial hemolymph parameters were measured for series C crabs. This group contained only uninfested and heavily infested crabs. Moderately infested crabs did not occur in this sample. Mean prebranchial and postbranchial hemolymph pH, PO_2 or C_{O_2} values for heavily infested crabs were not significantly

different from those values measured in uninfested crabs (Table 2-3) .

Arteriovenous (a-v) differences in hemolymph gas parameters for series C crabs were calculated for crabs in which samples of both prebranchial and postbranchial hemolymph had successfully been obtained (Table 2-4). Because of occasional problems with hemolymph clotting, we were not successful in measuring all parameters for every hemolymph sample. Uninfested and heavily infested crabs were not significantly different with respect to pH, C_{O_2} and P_{O_2} a-v differences.

Discussion

The presence of ectocommensal barnacles on crab gills could increase the crab's ventilatory dead space in several ways. Firstly, by cementing several gill platelets together upon attachment (Walker, 1974), the barnacle may prevent water circulation and thereby gas exchange between these platelets. As the barnacle grows, adult cement is secreted and this will fuse several more platelets (Walker, 1974). This should have a relatively minor effect due to the large number of platelets present in the gill chamber (12,400 on average for a 200 g blue crab; Gray, 1957). Secondly, barnacles remove oxygen from the water in the crab's ventilatory stream for their own metabolism. Although oxygen consumption rates for O. muelleri have not been measured, extrapolation from data for other barnacles such as Chthamalus

stellatus, C. depressus and Balanus perforatus (Hammen, 1972) at the same experimental temperature of 25°C would predict an $\dot{M}\ O_2$ of 0.9 - 1.7 nmol O_2 min^{-1} for each O. muelleri barnacle, which, even under heavy infestation, would be negligible relative to the $\dot{M}\ O_2$ of the crab (28 μmol kg^{-1} min^{-1}). Thirdly, the barnacle may create a physical obstruction in the ventilatory stream. The magnitude of this effect is difficult to estimate. An increase in ventilatory deadspace could lead to a simultaneous increase in perfusion deadspace because the hemolymph perfusing the poorly ventilated portion of the gills would not be as well oxygenated. Control of perfusion by vasoconstriction could prevent this from occurring, but such controls have not been described in crustaceans (McMahon and Wilkens, 1983).

A possible long-term effect is that the presence of the barnacle may inhibit the gill-cleaning action of the epipodites of the second and third maxillipeds (gill rakers) allowing other barnacles to attach and debris to build up (Walker, 1974). Many crustaceans periodically reverse the direction of ventilatory flow over their gills for several reasons, including removal of detritus and particulate matter from the gill chamber (McMahon and Wilkens, 1983). Reversal frequency increases in response to irritants (Batterton and Cameron, 1978), particulate matter (Arudpragasam and Naylor, 1964; Berlind, 1977), and hypoxia (Taylor et al., 1973). During reversed ventilation, oxygen uptake is not as effective (McDonald, 1977).

Respiratory values recorded here for uninfested crabs are generally similar to previously reported values for resting blue crabs (Batterton and Cameron, 1978; Aldridge and Cameron, 1979; Booth et al., 1982; Booth et al., 1984), although slightly lower $\dot{M}O_2$ and f_H values suggest that the crabs in this study may have been more "settled". In earlier studies using restrained crabs fitted with a respiratory mask, mean $\dot{M}O_2$ was 50 μmol $kg^{-1}min^{-1}$ and mean f_H was 89 beats min^{-1} (Booth et al., 1982) compared to 28 μmol $kg^{-1}min^{-1}$ and 74 beats min^{-1} in the present study. However, mean f_{SC} in this study (130 beats min^{-1}) was above that found previously (94 beats min^{-1} - Booth et al., 1982; 109 beats min^{-1} - Batterton and Cameron, 1978). The higher f_{SC} may be related to the PO_2 of the inhalent water, which was lower in this study (17 kPa, which was further lowered by mixing with exhalent water in the respiratory chamber) than in the earlier study (18 kPa - Booth et al., 1982). Exact correspondence between studies is not expected because of the effects of geographic (Mauro and Mangum, 1982) and seasonal variation (Weiland and Mangum, 1975), as well as temperature and salinity (Laird and Haefner, 1976) of acclimation and experimentation on hemocyanin-oxygen affinity (Mangum, 1983). In addition different measurement methods for $\dot{M}O_2$ (flow-through versus closed system respirometry and masked versus unrestrained animals), may explain small differences between studies.

It is not surprising that infested crabs maintain $\dot{M}O_2$ at the same levels as uninfested crabs. Trout maintain resting $\dot{M}O_2$ when up to 30% of their gill surface area has been cauterized (Duthie and Hughes, 1987). When spiders had their respiratory surface area halved by experimental manipulation they maintained oxygen consumption by doubling heart rate (Anderson and Prestwich, 1982).

The elevated f_H and f_{SC} rates found in the infested crabs in this study are greater than values reported for resting blue crabs (f_H = 89, f_{SC} = 94 beats min^{-1}; Booth et al., 1982), but not as great as those found in exercising blue crabs (f_H = 143, f_{SC} = 312 beats min^{-1}; Booth et al., 1982)

The hemolymph parameters for uninfested controls are also similar to reported values (Mangum and Weiland, 1975; Booth et al., 1984). The pH and C_{CO_2} hemolymph values in the present study exhibit similar trends to those found in f_H and f_{SC}. Values for uninfested crabs are similar to those reported in the literature for resting crabs. Moderately infested crabs have significantly lower pH and C_{CO_2}, but not as low as reported for exercising blue crabs (Booth et al., 1984). Pre- and postbranchial hemolymph pH values are not different from each other confirming the findings of Mangum and Weiland (1975) and Booth et al. (1984). The lactate values in the present study, although low (1.9 mM), are higher than reported in resting blue crabs (0.7-1.3 mM; Booth et al., 1982); as in that study no differences between pre- and postbranchial hemolymph lactate levels were found.

Variation exists in blue crab hemolymph PO_2 values in the literature. Booth et al. (1982) found a large arteriovenous PO_2 difference due to a high postbranchial value (10.4 kPa) and a low prebranchial PO_2 of 1.1 kPa. The present study reports postbranchial (4.7 kPa) and prebranchial (2.0 kPa) PO_2 values similar to Mangum and Weiland (1975). This is puzzling given that similar techniques were used in all three studies. However, the more extreme values (Booth et al., 1982) are very close to those reported for crabs stressed by exercise (Booth et al., 1982) and were reported as "routine" activity values rather than inactive "settled" values.

Ventilation volume, $\dot{V}_W$, and cardiac output, $\dot{V}_B$, and therefore gill perfusion volume, are directly related to f_{SC} and f_H, respectively (McMahon and Wilkens, 1983). The linear relationship between $\dot{V}_W$ and f_{SC} has been estimated (Batterton and Cameron, 1978). The 83% increase in f_{SC} measured in infested crabs predicts a 69% increase in $\dot{V}_W$ from 128 ml min^{-1} to 216 ml min^{-1} (for a 200 g crab). The Fick equation relates $\dot{V}_W$ or $\dot{V}_B$ (in ml min^{-1} kg^{-1}) to $\dot{M}_{O_2}$ (in μmol kg^{-1} min^{-1}) and the differences in oxygen contents between water or hemolymph entering and leaving the branchial exchanger (in μmol ml H_2O^{-1}).

$$\dot{V}_W = \frac{\dot{M}_{O_2}}{C_{IO_2} - C_{EO_2}}$$

$$\dot{V}_B = \frac{\dot{M}O_2}{CaO_2 - CvO_2}$$

In these equations CIO_2 and CEO_2 are the oxygen contents of the water entering and exiting the gill chamber, respectively, and CaO_2 and CvO_2 the oxygen contents of the arterial and venous hemolymph. Since $\dot{M}O_2$ remains constant between uninfested and infested crabs, and $\dot{V}_W$ and $\dot{V}_B$ both increase in infested crabs, then the CO_2 differences must decrease in infested crabs. This is true for $CaO_2 - CvO_2$ (Table 4); we do not have data for CIO_2-CEO_2. Decreases in $CIO_2 - CEO_2$ and $CaO_2 - CvO_2$ values indicate that the effectiveness of oxygen exchange has decreased. Given the calculated increase in $\dot{V}_W$ and the constancy of $\dot{M}O_2$ for infested and uninfested crabs, the Fick equation predicts that $CIO_2 - CEO_2$ decreases by 41%. The effectiveness of oxygen extraction from the ventilatory current ($\%Ext_W$):

$$\%ExtW = \frac{CIO_2 - CEO_2}{CIO_2} \quad (100)$$

would also decrease by 41% on average in infested crabs.

Increasing the cardiac output would increase the gill perfusion to compensate for increases in perfusion deadspace.

Cardiac output would be elevated by an increase in f_H and stroke volume. Using mean $\dot{M}O_2$ values from series A crabs (Table 1), mean a-v C_{O2} differences for series C crabs (Table 4) and the Fick equation, one can calculate a cardiac output ($\dot{V}_B$) of 354 ml kg^{-1} min^{-1} in uninfested crabs and 482 ml kg^{-1} min^{-1} in heavily infested crabs. These values are high relative to previously calculated values for resting blue crabs at the same temperature (Mangum, 1977; deFur and Mangum, 1979), but comparable (Booth et al., 1982). However, because the calculation is based on data from two different series of crabs, the absolute values should be viewed with caution. In relative terms, the calculated 1.4-fold increase in $\dot{V}_B$ correlates well with the 1.4-fold increase we measured in f_H.

By maintaining $\dot{M}O_2$, infested crabs should be able to maintain hemolymph blood gas variables at the same levels as maintained in uninfested crabs. The prebranchial hemolymph parameters suggest that moderately infested crabs were disturbed (acidosis, elevated PO_2, and decreased C_{CO2}) while heavily infested crabs were not. These are the same prebranchial hemolymph characteristics exhibited by blue crabs stressed by exercise (Booth et al., 1982; Booth et al., 1984). Moderately infested crabs are more likely to have been recently infested and may not have adjusted to the presence of barnacles. It is also possible that only individual crabs that are capable of compensating for the barnacles presence are able to survive a heavy infestation. Those crabs that were disturbed by a heavy

infestation would die and therefore not be sampled. Moderately infested individual crabs that are unable to compensate fully might still survive. Compensation for the barnacle-induced increase in ventilation and perfusion deadspace involves elevation of ventilation and perfusion rates as discussed above. However, increasing perfusion rate via elevated cardiac output would limit gas exchange in the metabolizing tissues. Successful compensation would include enhancement of tissue O_2 delivery via modification of hemocyanin oxygen binding characteristics (Mangum, 1983; Morris, 1990).

The comparison of the pre- and postbranchial hemolymph of infested and uninfested crabs showed that heavily infested crabs were able to maintain hemolymph variables at similar levels to uninfested crabs, confirming the results from the series B crabs.

Although one would expect Octolasmis muelleri to maximally exploit its host, the barnacle is dependent on the crab's ventilatory stream and continued survival and would therefore undergo natural selection to minimize any detrimental effect on its host. These results confirm that settled, infested crabs do not show signs of significant disturbance. However, crabs with massive infestations did not survive experimental handling and would probably not survive long in nature if stressed. Although the barnacle is common in Florida blue crabs, the median infestation level is not heavy (Gannon, 1990), thus it is unlikely that the barnacle poses a serious threat to the blue crab population.

Table 2-1. Mean whole-animal respiratory characteristics of series A Callinectes sapidus, infested (experimentals) and uninfested (controls) with Octolasmis muelleri. Values are expressed as mean ± S.E. (number of observations). The asterisk denotes significant differences between infested crabs and uninfested controls ($P < 0.05$).

	Uninfested	Infested
$\dot{M}O_2$ (μmol kg^{-1} min^{-1})	28.3 ±3.4 (8)	28.9 ±2.6 (14)
f_H (beats min^{-1})	73.8 ±11.2 (6)	106.5 * ±8.7 (9)
f_{SC} (beats min^{-1})	130.1 ±26.7 (14)	237.6 * ±30.8 (17)

Table 2-2. Mean prebranchial hemolymph parameters of series B Callinectes sapidus at three levels of infestation by Octolasmis muelleri. Values are expressed as mean ± S. E. (number of observations). Asterisks denote values that are significantly different from uninfested (control) crabs. ($P < 0.05$)

Infestation Level	pH	P_{O_2} (kPa)	C_{CO_2} (mM)	$[La^-]$ (mM)
Uninfested	7.50 ±0.05 (16)	1.96 ±0.27 (14)	6.0 ±0.4 (16)	1.9 ±0.1 (21)
Moderate	7.22* ±0.06 (7)	3.23* ±0.59 (6)	4.5* ±0.3 (6)	1.9 ±0.2 (3)
Heavy	7.52 ±0.05 (13)	1.93 ±0.35 (11)	6.6 ±0.6 (13)	1.9 ±0.1 (16)

Table 2-3. Mean prebranchial and postbranchial hemolymph parameters of series C Callinectes sapidus, uninfested (controls) and heavily infested with Octolasmis muelleri. Values are expressed as means ± S.E. (number of observations)

Infestation Level	Postbranchial pH	Postbranchial P_{O_2} (kPa)	Postbranchial C_{O_2} (μM)	Prebranchial pH	Prebranchial P_{O_2} (kPa)	Prebranchial C_{O_2} (μM)
Uninfested	7.50 ±0.13 (10)	4.71 ±0.96 (9)	178 ±178 (7)	7.56 ±0.14 (8)	2.68 ±0.68 (7)	71 ±99 (9)
Heavily infested	7.42 ±0.12 (10)	4.08 ±0.87 (8)	114 ±114 (10)	7.34 ±0.11 (10)	2.45 ±0.52 (10)	51 ±75 (9)

Table 2-4. Mean arteriovenous differences for hemolymph parameters of Callinectes sapidus, uninfested (controls) and heavily infested with Octolasmis muelleri. Values are expressed as mean ± S.E. (number of observations).

Infestation Level	pH	P_{O_2} (kPa)	C_{O_2} (μM)
Uninfested	-0.06 ±0.17 (8)	0.31 ±0.31 (6)	81 ±120 (6)
Heavily Infested	0.05 ±0.16 (9)	1.64 ±0.68 (7)	62 ±75 (8)

CHAPTER 3
EFFECTS OF *Octolasmis muelleri* INFESTATION ON EXERCISING AND RECOVERING BLUE CRABS

Introduction

Previous reports on symbionts of commercially important species such as the blue crab have focused on crab death and debilitation (Overstreet, 1978) or transmission of disease to humans (Moody, 1982). Natural selection generally acts on symbionts to minimize damage to their host but also to maximize the benefit they derive from the host. These opposing forces create an evolutionary dilemma for commensal species. How this dilemna is resolved is of theoretical importance. The effects of gill parasites and commensals on crustacean host gas exchange have been largely ignored. Branchial symbionts could impair host respiration in several ways: by removing oxygen from the ventilatory stream, through damaging branchial tissue, through obstructing the ventilatory stream, and/or inhibiting the ventilatory pumping mechanism.

The ectocommensal gill barnacle, Octolasmis muelleri Coker, does not feed on the tissues of its host crab (Walker, 1974). The oxygen uptake of this barnacle is presumed to be negligible (See Chapter 2) and it does not normally inhibit the host's pumping appendage, the scaphognathite. However, heavily infested crabs are more likely to die during handling and aerial exposure, suggesting that the ectocommensal causes physiological stress to the host (Gannon and Wheatly, 1988). In the previous chapter I demonstrated that this barnacle stimulates hyperventilation and tachycardia in its host Callinectes sapidus, and in moderate infestations, causes decreased prebranchial hemolymph pH and PO_2 and increased CO_2. Presumably these effects arise from obstruction of the host ventilatory stream. These physiological effects are similar to the typical respiratory responses to exercise.

The purpose of this phase of the study was to examine in detail the effects of O. muelleri infestation on the respiration of the blue crab during exercise, a natural stress. In nature, blue crabs are extremely active, swimming at speeds up to 1 m sec^{-1} (Spirito, 1972), and with well documented long range swimming migrations (up to 500 km; Oesterling and Adams, 1982). The effect of exercise on respiration has been well studied in decapod crustaceans (see reviews by McMahon, 1981; Wilkens, 1981) and particularly in blue crabs (Booth et al., 1982; Booth et al., 1984; Milligan et al., 1989).

The aerobic and anaerobic capacities of exercising decapods are comparable to those of lower vertebrates of the same size and activity level (Booth et al., 1982). Maximal oxygen consumption rates (McMahon, McDonald and Wood, 1979; Rutledge, 1980; Booth et al., 1982) are less than those reported for active fish (Brett, 1972) but greater than those reported for sluggish fish (Brett and Blackburn, 1978; Poulson, 1963). Decapods have been studied swimming (Booth et al., 1982), tail flipping (Rutledge, 1980), walking on treadmills (terrestrial; Wheatly, McMahon, Burggren and Pinder, 1985), and walking on substrate (aquatic; McDonald, McMahon, and Wood, 1979) with similar results. Generally, during exercise oxygen transport and delivery are enhanced through increased oxygen uptake, facilitated by increases in ventilation, perfusion, and the oxygen tension gradient across the gills (McMahon, 1981). The hemolymph respiratory pigment (hemocyanin) assumes a proportionally greater role in oxygen delivery to the tissues (McMahon et al., 1979). Oxygen conductance across the gills may also increase (McMahon and Wilkens, 1983). The level of unused oxygen in the hemolymph (venous reserve) decreases (Wood and Randall, 1981). The hemolymph acidosis is predominantly respiratory with a metabolic component in terrestrial crabs (Smatresk, Preslar and Cameron, 1979). The decreased pH causes a Bohr shift, which decreases hemocyanin-oxygen affinity thus facilitating oxygen delivery to the tissues.

Exercising blue crabs follow the pattern observed in the decapods but seem better adapted for aerobic exercise (Booth and

McMahon, 1985; Milligan et al., 1989). Swimming blue crabs increase ventilation, heart rate, and oxygen uptake so drastically and rapidly that maximum metabolic rate (227 μmol O_2 kg^{-1} min^{-1}) is larger than that reported for any other crustacean (McMahon and Wilkens, 1983). Aerobic metabolic scope measurements for blue crabs range from 2.6 X (Booth et al., 1982) to 3.4 X (McMahon and Wilkens, 1983). These values are lower than actual metabolic scope because the resting values used in these calculations were routine metabolic rates rather than quiescent rates. If the resting oxygen consumption rates measured in the present study (Chapter 2) had been used in these calculations, aerobic metabolic scope would be 4.6 - 8.1 X.

Blue crabs will swim continuously for over an hour in experimental conditions (Booth et al., 1982) resulting in a hemolymph acidosis that is mainly (80%, Booth et al., 1984) metabolic (i. e. due to metabolic acids in the blood rather than elevated CO_2 in the blood) and elevated lactate levels (Booth and McMahon, 1985). The acidosis effect is limited by the efflux of H^+ at the gills (Milligan et al., 1989). Lactate counteracts the effect of lowered pH on hemocyanin-oxygen affinity (Truchot, 1980, Booth et al., 1982). Recovery takes several hours (Milligan et al., 1989).

Because resting blue crabs infested with Octolasmis muelleri compensate for the barnacles' presence by elevating ventilation and heart rates (Gannon and Wheatly, 1988, See

Chapter 2), one would expect their ability to further increase these parameters during exercise to be compromised.

Methods

Animal Maintenance

Adult blue crabs (150 - 250 g) were collected in commercial traps at the University of Florida Marine Laboratory at Seahorse Key on the Gulf coast of Florida (29° 06' N. lat., 83°04' W. long.) from December 20, 1988 to October 5, 1989. Crabs were held at densities of less than 30 in an 800 l tank of recirculating filtered seawater (33-36 ppt salinity) at 22-26°C on a 12 hr light:12 hr dark cycle at the University of Florida in Gainesville. The crabs were fed chopped fish 1-2 times weekly, but food was withheld 48 hours prior to experimentation. Intermolt crabs (stage C-4, Johnson, 1980) were given a minimum of one week to acclimate to these conditions before use in experiments.

Experimental Protocol

Ventilation rate (f_{SC}), heart rate (f_H) and prebranchial (venous,v) and postbranchial (arterial,a) hemolymph characteristics (pH; oxygen tension, PO_2; oxygen content, CO_2; and (L+)-lactate concentration, $[La^-]$) were measured in 39 crabs. Hemolymph samples were withdrawn for analysis from all crabs after at least 24 hours of isolation (resting), at the end of a 15 minute exercise period, and one hour after recovery under settled conditions. The f_{SC}

and f_H were recorded for one hour near the end of the resting period and continuously during the exercise and recovery periods. The animals sampled included crabs that were naturally infested by the ectocommensal barnacle Octolasmis muelleri and controls that were not infested. This was a blind experiment. Infestation level could only be determined after experimentation. After measurements were completed, crabs were killed by freezing and the gill chambers were opened and visually inspected for the presence of Octolasmis muelleri. The total mass of barnacles present in the gill chamber of each crab was used as an index of infestation level.

Analytical Techniques

Experimental crabs were placed in a nalgene® holding tank containing 38 l of filtered seawater (33-36 ppt salinity) at 23-25°C. Ventilation rate, f_{SC}, measured as frequency of scaphognathite beating, and f_H were measured using an impedance conversion technique (Dyer and Uglow, 1977). Each crab was prepared by drilling two holes through the carapace on either side of the heart. The stripped end of a lacquered copper wire (32 gauge) was inserted into each hole to a depth of 4 mm. The wires were held in place with cyanoacrylate glue and latex dental dam. These wires were attached to an impedance convertor (UFI 2991) and the output was recorded on a Grass model 79 polygraph. Scaphognathite rate was recorded similarly, with a recording wire inserted into the exhalent canal of each branchial chamber and a reference wire attached to the carapace directly above each scaphognathite.

Prebranchial hemolymph samples (1 ml) were taken from the infrabranchial sinus by penetrating the arthrodial membrane at the base of a swimming leg with a 22 guage needle on a chilled syringe. Crabs were prepared for postbranchial hemolymph sampling by drilling a hole through the carapace directly over the heart, through the carapace but not penetrating the epidermis. This hole was sealed with a 1 cm^2 patch of latex dental dam and cyanoacrylate glue. The postbranchial hemolymph sample (1 ml) was withdrawn by puncturing the dental dam patch. The crabs were given 48 hours to recover from this surgery before experimentation.

The C_{O_2} of an 80 µl subsample of each hemolymph sample was analyzed in a Lex-O_2-con oxygen analyzer. The [La^-] of an 80 µl subsample was measured using a commercial reagent kit (Sigma #826). An 840 µl subsample was injected into an IL 213 blood gas analyzer thermostatted to 25°C. The PO_2 of this subsample was measured with an IL O_2 electrode (20984) and the pH was measured with an IL pH electrode (20982).

After surgery crabs were placed in a harness of plastic coated baling wire which was looped around the lateral horns of the carapace and attached to a 25 cm long (1 cm diameter) wooden dowel. The dowel was attached by a clamp to a wooden pole suspended over the nalgene holding tank. This arrangement made it possible to hold the crab either on the floor of the tank or suspended in the water column with minimal restraint. When crabs were raised off the floor of the holding tank they would swim continuously. Infrequently crabs required prodding with another

wooden dowel in order to continue swimming. Hemolymph samples were taken from crabs after 24 hours of isolation on the floor of the holding tank (resting), after fifteen minutes of swimming in the water column (exercise), and after one hour of recovery on the floor of the holding tank (recovery). A period of fifteen minutes was selected for the exercise period because, in earlier studies on blue crabs, (Booth et al., 1982, Booth et al., 1984) physiological variables showed maximal change and reached or approached peak levels in the first fifteen minutes of exercise.

Data Analysis and Statistics

Experimental crabs were divided into three classes based upon intensity of infestation: uninfested (0.0 - 0.020 g of Octolasmis muelleri in the crab gill chambers), moderately infested (0.020 - 0.100 g of O. muelleri), and heavily infested (0.100 - 1.22 g of O. muelleri). These divisions were based on an analysis of the distribution of O. muelleri on 512 blue crabs (See Chapter 1). Mass of infesting barnacles was assumed to be a more accurate index of potential barnacle impact than number of infesting barnacles. In experiments described in Chapter 2, however, numerous crabs were examined on a single day and it was impossible to carefully remove all of the infesting barnacles and weigh them for each crab.

The continuous records of f_{SC} and f_H were analyzed by counting the number of beats in a fifteen second interval at the beginning of every minute. These values were expressed as beats min^{-1} and combined into five minute means. For comparison of

crabs at the different infestation levels, the mean frequency of the last fifteen minutes of the acclimation period was used for the "resting" value. The mean of the final five minute interval of the exercise period was used for the "exercise" value and the mean of the last five minutes interval of the recovery period was considered the "recovery" value. After variances were found to be homogeneous with an F-test (Sokal and Rohlf, 1969), heart and ventilation rates of crabs at the different infestation levels were each compared with a one-way factorial ANOVA (Winer, 1971). Heart rate and ventilation rate values during the full one hour recovery period were analyzed with repeat measures ANOVA (Winer, 1971) to determine if a significant change occurred in either of these parameters during the recovery period.

Pre- and postbranchial blood chemistry measurements were also compared with repeat measures ANOVA to determine significance of any differences between the rest, exercise and recovery periods. Prebranchial values were compared to postbranchial values with paired t-tests to determine if a-v differences were significant. Hemolymph chemistry data were reported as mean + S.E. These values were also compared across infestation levels with a factorial ANOVA. Hemolymph pH values were converted to concentration (linear scale) before calculation of means, S.E.s and ANOVAs.

Results

To minimize any effect of mass on heart rate (f_H) and ventilation rate (f_{SC}), I used crabs of similar mass (mean = 249 ± 45.2 g SD; range = 128 - 343 g). However, f_H and f_{SC} were plotted against mass for all crabs and for the crabs at each infestation level separately and subjected to regression analysis. No linear relationships were found, indicating that the effect of mass was minor over this mass range.

At rest, f_H for heavily infested crabs was significantly greater than f_H for uninfested crabs (factorial ANOVA; $F_{2,30} = 2.4$; Fisher PLSD; $P < 0.05$). The heart rates of crabs at the three levels of infestation (figure 3-1) all increased dramatically during exercise from resting rates of around 75 - 100 beats min^{-1} up to 140 - 160 beats min^{-1} remaining at this level throughout the exercise and recovery periods. This represented a 2.0 X increase for uninfested crabs and a 1.4 X increase for heavily infested crabs. Heart rates for the three groups of crabs were not significantly different through the exercise and recovery periods. At the end of the one hour recovery period, f_H for all three groups of crabs was not significantly different from f_H at the beginning of the recovery period (repeat measures ANOVA, $P > 0.05$).

Ventilation rates (fig. 3-2) followed a similar pattern for resting and exercising crabs. At rest, heavily infested crabs had significantly greater f_{SC} than moderately infested and uninfested crabs (factorial ANOVA, $F_{2,29} = 8.3$; Fisher PLSD, $P < 0.05$).

Variance in ventilation rates was much greater for exercising crabs than for resting crabs and differences between infestation levels were not significant. As exercise began, f_{SC} increased from about 100 beats min^{-1} to about 300 beats min^{-1} in all three groups. Unlike heart rate, ventilation rate began to decrease immediately after exercise stopped, but as f_H, did not reach resting levels by the end of the defined recovery period. Ventilation rates at the end of the recovery period were significantly lower than f_{SC} at the onset of the recovery period for all three groups of crabs (repeat measures ANOVA; $F_{3,44} = 83.9$; $P < 0.05$).

The frequency of ventilatory pauses (figure 3-3) was significantly greater for uninfested crabs than moderately or heavily infested crabs (factorial ANOVA; $F_{2,31} = 3.3$; Fisher PLSD; $P < 0.05$) at rest. During exercise, ventilatory pauses were not observed in any crabs. In the recovery period few crabs exhibited ventilatory pauses and no differences existed between the groups of crabs.

The pre- and postbranchial hemolymph oxygen contents (C_{O2}) for resting, exercising and recovering crabs at the three infestation levels (figure 3-4) were compared with a 2-way repeat measures ANOVA (Winer, 1971). The repeat measures gave a significant effect ($F_{5,10} = 11.5$; $P < 0.05$). Means were compared with the Fisher PLSD (Snedecor and Cochran, 1980). The mean recovery postbranchial value was significantly greater than the resting and exercising prebranchial means ($P < 0.05$). This difference was most pronounced in the uninfested

crabs. The C_{O_2} values of uninfested, moderately or heavily infested crabs were not significantly different from each other.

For uninfested crabs the mean C_{O_2} a-v difference increased from a resting value of 0.042 mM to a recovery value of 0.091 mM. In heavily infested crabs the mean C_{O_2} difference increased from a resting value of 0.079 mM to a recovery value of 0.22 mM. In moderately infested crabs the mean C_{O_2} a-v difference decreased from 0.092 mM to 0.054 mM. During the recovery period heavily infested crabs had a significantly greater a-v difference than moderately and uninfested crabs (factorial ANOVA; $F_{2,25} = 4.2$; Fisher PLSD; $P < 0.05$).

Because the 2-way repeat measures ANOVA could not include individuals with a missing measurement in the calculations (Winer, 1971), and some samples were lost due to blood clots, this test could not be used for PO_2 comparisons. Six separate1-way repeat measures ANOVA were used to compare pre- and postbranchial hemolymph PO_2 for crabs at each infestation level over the rest, exercise and recovery periods (figure 3-5). Only the difference between resting prebranchial PO_2 and recovery prebranchial PO_2 for uninfested crabs was significant (repeat measures ANOVA; $F_{2,23} = 3.9$; $P < 0.05$). Comparison of the PO_2 levels of crabs at the different infestation levels (factorial ANOVA) were made for pre- and postbranchial hemolymph at the three experimental periods. No significant differences were found.

The a-v PO_2 differences for uninfested and moderately infested crabs during rest, exercise and recovery were not significant (paired t-test). Heavily infested crabs had small a-v differences at rest, but the mean PO_2 a-v difference for heavily infested exercised crabs (1.2 kPa) was significantly greater (factorial ANOVA; $F_{2,23} = 3.9$; $P < 0.05$) than the mean PO_2 a-v difference for uninfested exercised crabs (0.25 kPa) and moderately infested crabs (0.09 kPa).

The trend for hemolymph pH values for all crabs was to decrease during exercise and increase during recovery, but not to resting levels (figure 3-6). A two-way repeat measures ANOVA was used to compare pre- and postbranchial hemolymph values of resting, exercising and recovering crabs at the three infestation levels. The repeat measures gave a significant effect ($F_{5,10} = 6.9$; $P < 0.05$). Mean pre- and postbranchial pH values during exercise were significantly lower than resting values (Fisher PLSD; $P < 0.05$). Samples for several crabs were lost due to blood clotting. To include these individuals in comparisons, I used six one-way factorial ANOVA comparisons of pre- and postbranchial hemolymph pH values for crabs at the different levels of infestation during rest, exercise and recovery. No significant differences were found between infestation levels.

The moderately and heavily infested crabs had a significantly greater postbranchial hemolymph pH than prebranchial pH (paired t-test; $P < 0.05$) during rest, exercise

and recovery. Uninfested crabs had a significant a-v difference only during rest.

Hemolymph lactate concentrations showed the reverse of the trend described for pH (figure 3-7). A 2-way repeat measures ANOVA was used to compare pre- and postbranchial lactate values of resting, exercising and recovering crabs at the three infestation levels. Mean lactate increased significantly during exercise and decreased significantly during recovery, but not to resting levels ($F_{2,19} = 25.6$; $P < 0.05$). Differences between pre- and postbranchial lactate were not significant, with the exception of the recovery values for the heavily infested crabs (paired t-test; $P < 0.05$). Differences between the crabs at the three infestation levels were not significant.

Discussion

The mean heart rate of uninfested crabs at rest (78.4 beats min^{-1} ± 5.6 S.E.) agrees with that found earlier for uninfested blue crabs (73.8 beats min^{-1} ± 11.2 S.E.) (See Chapter 1). In the prior study, infested crabs (most of which would be in the heavily infested category in this study) had an elevated mean resting f_H (106.5 beats min^{-1} ± 8.7 S.E.) that also agrees with mean resting f_H in the heavily infested crabs in this study (103.6 beats min^{-1} ± 8.7 S.E.).

These f_H values for uninfested crabs are lower than those found by Booth et al. (1982) for resting blue crabs (89 beats min^{-1} $\pm$ 9.0 S.E.). However, that study of Florida gulf coast blue crabs may have included a mix of infested and uninfested blue crabs since the underside of the gills were not inspected for infesting barnacles (B. R. McMahon, personal communication) and infestation levels in the Cedar Key, Florida crab population average 40% (See Chapter 1). Another reason for an elevated heart rate among the blue crabs in Booth's study was that they were fitted with a respiratory mask and were restrained during measurement. Their resting values are labelled "routine" rather than standard.

Ventilation rates differ from those found by Booth et al. (1982) in the same way. Resting moderately infested crabs had the same f_{SC} as resting blue crabs in the earlier study (93 beats min^{-1}) with uninfested blue crabs having a significantly lower f_{SC} and heavily infested crabs a significantly higher f_{SC}. These resting heart and ventilation rates for blue crabs are higher than those found for similar sized crab species (McMahon and Wilkens, 1977; McMahon,McDonald and Wood, 1979); however few of these species are as active as the blue crab (McMahon, 1981).

Crabs at all three infestation levels exhibited an immediate elevation of heart and ventilation rates to near maximum as exercise began and maintained elevated levels throughout the exercise and recovery periods. Booth et al. (1982) found the same rapid increase, with a steady state half time of about 30 seconds. Exercise f_H and f_{SC} values found by Booth et al. (1982) were

virtually the same as those found in moderately infested crabs in the present study, and were below values found in uninfested crabs. Although the exercise period in the earlier study was one hour (compared with the present exercise period of fifteen minutes) the peak levels reached and the dynamics of recovery were similar. Ventilation rate dropped abruptly as exercise stopped. Heart rate did not decrease significantly but remained high. Neither rate reached resting values after thirty minutes of recovery in either study. Even after one hour, recovery was not complete in the present study.

Although differences in heart rate between the three infestation levels were not significant, uninfested crabs had greater mean f_H and f_{SC} over all five-minute exercise and recovery intervals but one. Variance was great during exercise and recovery, particularly for f_{SC}. Although heavily infested crabs were hyperventilating at rest, they were able to elevate f_{SC} during exercise to about the same levels as uninfested crabs, perhaps because this level (300 - 350 beats min^{-1}) is the maximal sustainable rate. Batterton and Cameron (1978) measured ventilation volume in blue crabs over a large range of f_{SC} but could not stimulate f_{SC} above 350 beats min^{-1}. Values of f_{SC} over 300 beats min^{-1} are more than twice as great as the values reported for other exercising crabs (McMahon et al., 1979; Wood and Randall, 1981) or crayfish (Rutledge, 1980; Booth and McMahon, 1980) indicating the great aerobic exercise capacity of blue crabs.

Intermittent cardiac and ventilatory pauses are characteristic of decapod crustaceans at rest in well-oxygenated undisturbed environments (McDonald et al., 1979; McMahon and Wilkens, 1977; Batterton and Cameron, 1978). Although experimental evidence is lacking, these pauses may be a way of conserving energy when hemolymph oxygen levels are near or above saturation levels (McMahon and Wilkens, 1977). At rest, uninfested crabs ceased ventilating more than once every two minutes. Heavily infested crabs paused in their ventilation only once every twenty minutes. During exercise oxygen demand increases and ventilatory pauses were not observed.

Cardiac pauses were more difficult to evaluate. Frequently, ventilatory and cardiac pauses would occur simultaneously. While ventilatory pauses might last over a minute, single cardiac contractions would typically occur intermittently throughout the ventilatory pause, making calculation of the length or number of cardiac pauses difficult.

Resting and exercising hemolymph oxygen contents of crabs did not differ between infestation levels, but mean values were only about 20% of those found by Booth et al. (1982). They found C_{vO_2} and C_{aO_2} were not significantly changed after 25 minutes of exercise. I found the same result in moderately infested crabs. Uninfested crabs exhibited the same trend as heavily infested crabs; C_{aO_2} increased with exercise and recovery. Since C_{vO_2} did not significantly change, the C_{O_2} a-v differences increased, and the recovery a-v difference was greatest for heavily infested

crabs. This suggests that the metabolizing tissues of heavily infested crabs are not reducing prebranchial CO_2 below the levels of uninfested crabs, but they are obtaining more oxygen because the a-v difference is greater.

Resting prebranchial hemolymph oxygen tensions for all groups of crabs were low, but agree with those reported for uninfested resting blue crabs in Chapter 2 (Mangum and Weiland, 1975; Booth et al., 1982). Resting postbranchial PO_2 values however were about 20-50% less than those reported by Mangum and Weiland (1975) and Booth et al. (1982). Differences between post- and prebranchial hemolymph PO_2 reported by Booth et al. (1982) were large for crabs at rest (59 torr, 7.9 kPa) and during exercise (68 torr, 9.1 kPa). In this study, uninfested and moderately infested crabs had virtually the same PO_2 for post- and prebranchial hemolymph during the rest, exercise and recovery periods. The a-v differences found in the heavily infested crabs during exercise and recovery are comparable to those reported by Mangum and Weiland (1975) for resting (ΔPO_2 = 2.72 kPa) and exercising (ΔPO_2 = 1.76 kPa) blue crabs.

Post- and prebranchial pH followed the pattern expected for exercising animals. Both values decreased during exercise and increased during recovery. Values for uninfested crabs were similar to those reported for blue crabs at rest and exercise (Mangum and Weiland, 1975; Booth et al., 1982; Booth and McMahon, 1985). Moderately infested crabs had lower resting pH values, but higher exercise values. Recovery values were not

available for other studies, but recovery values here were between resting and exercise levels for all three groups of crabs, indicating that recovery was not complete in one hour.

Respiratory acidosis accounted for less than 20% of the total pH drop in exercising blue crabs (Booth and McMahon, 1981). Although lactate and H^+ are produced in equimolar amounts in anaerobic glycolysis (Hochachka and Mommsen, 1983), Booth et al. (1984) calculated a net H^+ deficit in the hemolymph of exercising crabs. However, measured H^+ excretion into the external medium exceeded that predicted from lactate accumulation (Booth et al., 1984). Here, lactate levels in the hemolymph of resting crabs were similar to those reported for resting blue crabs (Booth et al., 1982; Milligan et al., 1989). During exercise, $[La^-]$ increased 2-4 X for crabs at all infestation levels. This is significantly less than the increases reported for blue crabs after exercise bouts of fifteen minutes (5 X-Booth et al., 1984), 25 minutes (14 X-Booth et al., 1982), and thirty minutes of exercise (10 X-Booth and McMahon, 1985; 15 X-Milligan et al., 1989). In determining lactate the samples were not buffered with EDTA to prevent interference by Cu^{2+} in the hemolymph as recommended by Engel and Jones (1978). However, this would equally affect all readings, preserving the ratio between treatments.

In blue crabs, anaerobic metabolism is important to energy production during the first few minutes of swimming, while sustained exercise is fueled predominantly by aerobic metabolism (Booth and McMahon, 1985). In some terrestrial decapods such as

Uca pugilator (Herreid, 1981) and Gecarcinus lateralis (Full and Herreid, 1984) anaerobic metabolism is important throughout exercise bouts. Energy expenditure during exercise is difficult to quantify. Earlier studies of blue crabs used prodding to induce exercise - walking and swimming (Mangum and Weiland, 1975; Booth et al., 1982; Booth et al., 1984). Intensity of exercise was not controlled in those studies nor in the present one. The crabs in this study all swam readily with little prodding once they were lifted from the substrate. However there was great variation in the rapidity of swimming leg movement. Subjective evaluation of swimming intensity did not reveal any correlation with infestation level. The lower lactate levels found in all three groups of exercising crabs indicate anaerobic glycolysis was not used to the same extent as by crabs in earlier studies (Booth et al., 1982; Booth et al., 1984). Perhaps overall energy expenditures here were less, resulting in lower lactate production and minimal disturbance of blood chemistry.

During exercise the decrease in hemolymph pH affects the hemocyanin-oxygen dissociation curve. Using the Bohr shift values calculated for blue crab hemolymph by Booth et al. (1982):

$$-1.14 = \frac{\Delta \log P_{50} \text{ (torr)}}{\Delta \text{ pH}}$$

I found the difference between post- and prebranchial hemolymph pH in heavily infested crabs during exercise equivalent to an increase in P_{50} of 1.4 torr (0.19 kPa). For moderately infested and uninfested crabs, the P_{50} increased 1.7 torr (0.23 kPa) and 0.0 torr respectively.

In blue crabs lactate concentration of the hemolymph affects P_{50} independent of pH (Truchot, 1980). The effect of increased hemolymph lactate on hemocyanin oxygen-affinity was measured *in vivo* and *in vitro* by Booth et al. (1982) as;

$$P_{50}\ (\text{torr}) = -5.252 \log [La^-] + 11.859$$

Using the mean $[La^-]$ found in the post- and prebranchial hemolymph of heavily infested crabs to calculate P_{50} values, I found a change in P_{50} from postbranchial to prebranchial hemolymph of -0.85 torr (-0.11 kPa), a negative Bohr shift. For moderately infested and uninfested crabs, lactate levels would shift the P_{50} -0.16 torr (-0.02 kPa) and -0.07 torr (-0.01 kPa) respectively. Because these pH and [lactate] values and the equations were taken from different studies and the present study did not correct for the possible interference of Cu^+ (Engel and Jones, 1978), these absolute values are only approximations. However, the relative effects on the hemocyanin-oxygen affinity relationship should hold. Thus, during exercise the pH decrease in prebranchial hemolymph would tend to shift the oxygen dissociation curve to the right, reducing oxygen loading at the

gills and increasing oxygen unloading at the tissues. However, this is opposed by the effect of increased lactate levels, which would tend to shift the curve to the left by almost the same amount for heavily infested crabs and by a lesser amount for moderately infested and uninfested crabs.

At the end of the 1-hour recovery period, neither pH nor [La^-] have reached recovery levels but because of the opposing effects of these two moderators, the P_{50} and therefore the position of the oxygen dissociation curve is relatively unchanged. This is an example of enantiostasis (Mangum and Towle, 1977). Lactate produced by anaerobic metabolism counteracts the negative effects of acidosis on hemocyanin-oxygen affinity (Milligan et al., 1989). Exercise in the blue crab is highly aerobic (Milligan et al., 1989) and the lactate effect preserves the "venous reserve" by maintaining unused oxygen in the prebranchial hemolymph that can be utilized when the intensity of exercise must be increased (Booth et al., 1984) or some other environmental stress demands emergency O_2 transport. During exercise, the actual amount of oxygen available to the tissues is determined by the hemocyanin-oxygen affinity which is affected by pH and [lactate] (Booth et al., 1982) as well as organic ion modulators (Morris, 1990). The fact that prebranchial oxygen content remains relatively unchanged during rest, exercise and recovery indicates that all groups of crabs are maintaining a venous reserve. Uninfested crabs had a significant a-v pH difference only at rest, while the prebranchial hemolymph pH of moderately and heavily infested crabs was

significantly less than the postbranchial hemolymph pH during rest, exercise and recovery. This suggests that moderately and heavily infested crabs relied more on the Bohr effect to deliver oxygen to their metabolizing tissues.

After 1 hour of recovery, f_{SC} had dropped significantly, while f_H remained elevated. The same relationship was observed by Booth et al. (1982) and McMahon et al. (1979), although f_{SC} and f_H are usually coordinated in their activity (Wilkens, 1981). During recovery $\dot{M}O_2$ dropped at about the same rate as f_{SC} (McMahon et al., 1979; Booth et al., 1982). This suggests that the demand for O_2 had decreased considerably. A decreased f_{SC} could still meet the decreased O_2 need. However lactate and pH values had not reached recovery levels at one hour of recovery. Perfusion rates would need to be maintained at a high level to flush La^- and H^+ from the muscle tissues. Excretion of H^+ is presumably by branchial exchange (Booth et al., 1984) while lactate is taken up and metabolized by tissues that have not been identified (Milligan et al.,1989).

Blue crabs have a well developed anaerobic capacity (Booth and McMahon, 1985) and a large aerobic metabolic scope (Booth et al., 1982). They are highly resistant to fatigue from sustained swimming (Milligan et al., 1989) maintaining a level of unused oxygen in their prebranchial hemolymph even after one hour of continuous swimming (Booth et al., 1982). The presence of the ectocommensal barnacle, Octolasmis muelleri in the gill chamber does not cause a major disturbance even during the stress of

exercise. At rest, infested crabs elevate f_{SC} and f_H to maintain a venous reserve and hemolymph variables at the levels of uninfested crabs, thereby compensating for the physical obstruction within the ventilatory stream. During sustained exercise f_{SC} and f_H for all crabs approach maximum rates. Oxygen delivery to the tissues is maintained, but oxygen uptake at the gills is not compromised due to the effect of lactate counteracting acidosis.

The physiology of the blue crab is extremely well designed for sustained swimming (Booth et al., 1982). The enantiostatic control of its oxygen delivery system (Mangum and Towle, 1977) makes Octolasmis-infested blue crabs capable of sustained swimming with minimal disturbance in their blood gas parameters. Because energy expenditure while swimming was not quantified, infested crabs may not have been swimming as fast as were uninfested crabs, and that extremely rapid escape swimming may be compromised by massive barnacle infestation. Several crabs with infestation of more than 1.0 g Octolasmis muelleri did not survive experimental stress, and probably would not survive long in nature.

Figure 3-1. Mean heart rates of resting, exercising and recovering crabs at three levels of infestation. Exercise and recovery periods are of 5 minute duration.

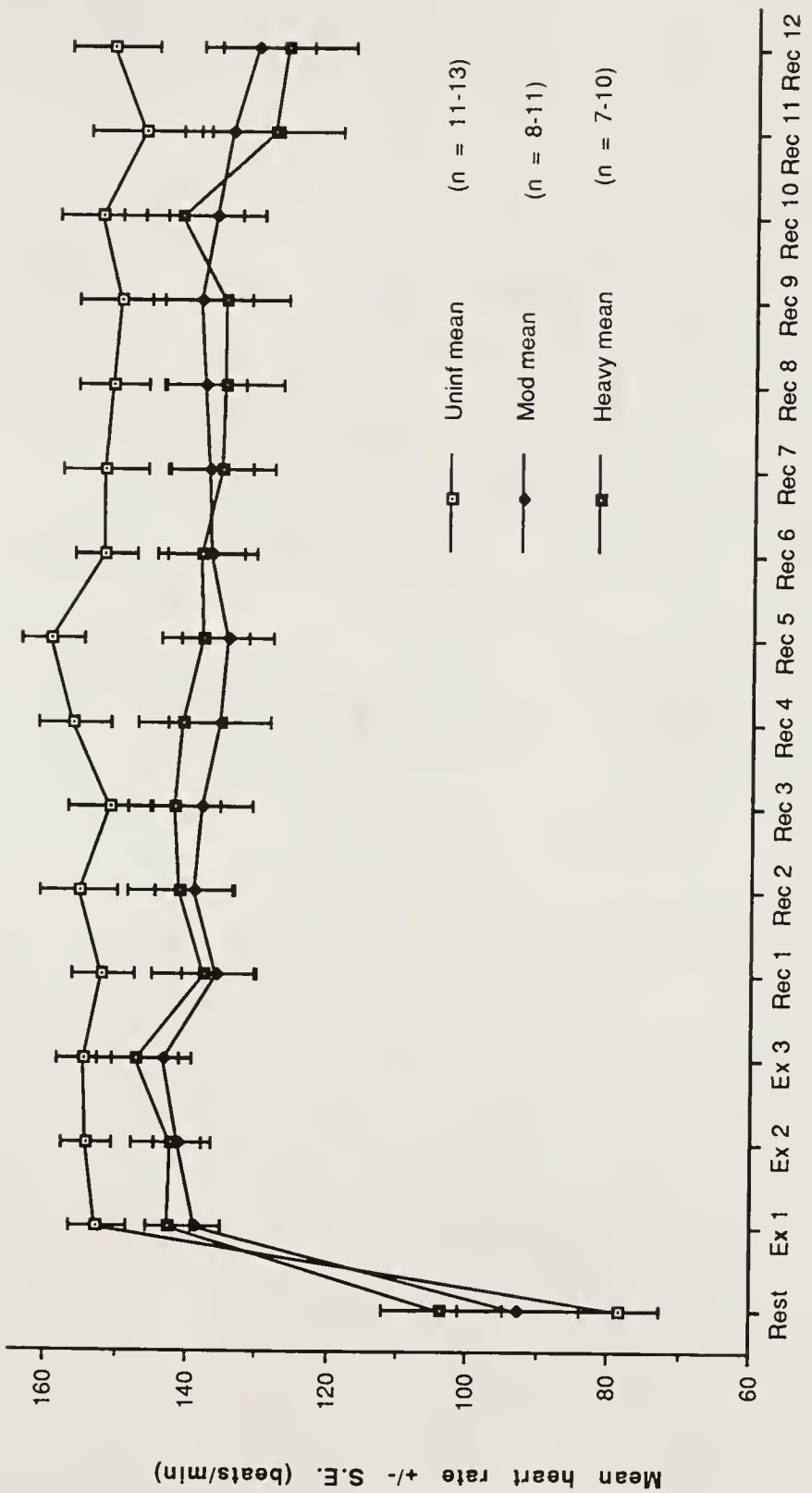
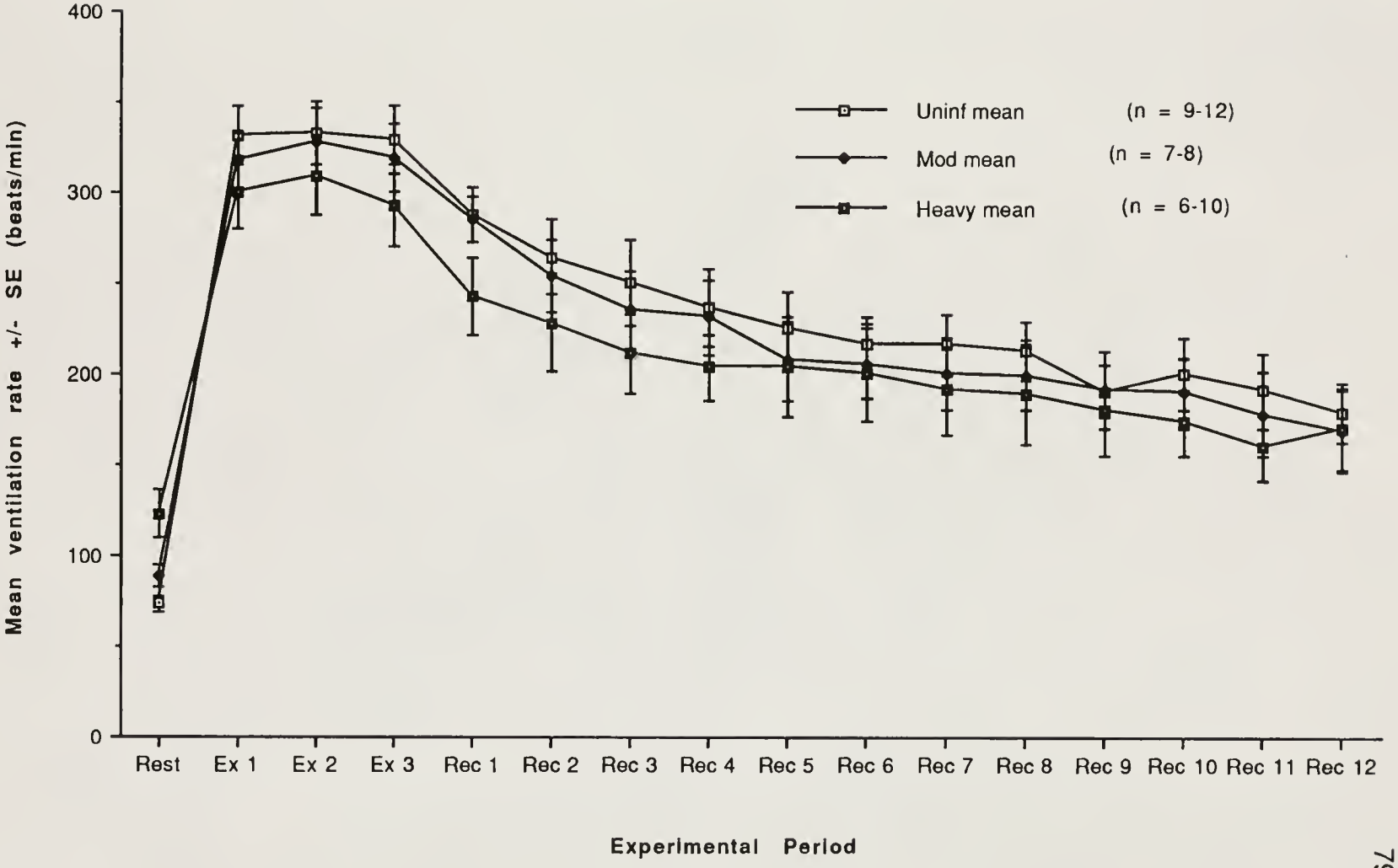

Mean heart rate +/- S.E. (beats/min)
160
140
120
100
80
60
Rest
Ex 1
Ex 2
Ex 3
Rec 1
Rec 2
Rec 3
Rec 4
Rec 5
Rec 6
Rec 7
Rec 8
Rec 9
Rec 10
Rec 11
Rec 12
Experimental Period
Uninf mean (n = 11-13)
Mod mean (n = 8-11)
Heavy mean (n = 7-10)

Figure 3-2. Mean ventilation rates of resting, exercising and recovering crabs at three levels of infestation. Exercise and recovery periods are of 5 minute duration


Mean ventilation rate +/- SE (beats/min)
400
300
200
100
0
Uninf mean (n = 9-12)
Mod mean (n = 7-8)
Heavy mean (n = 6-10)
Rest
Ex 1
Ex 2
Ex 3
Rec 1
Rec 2
Rec 3
Rec 4
Rec 5
Rec 6
Rec 7
Rec 8
Rec 9
Rec 10
Rec 11
Rec 12
Experimental Period

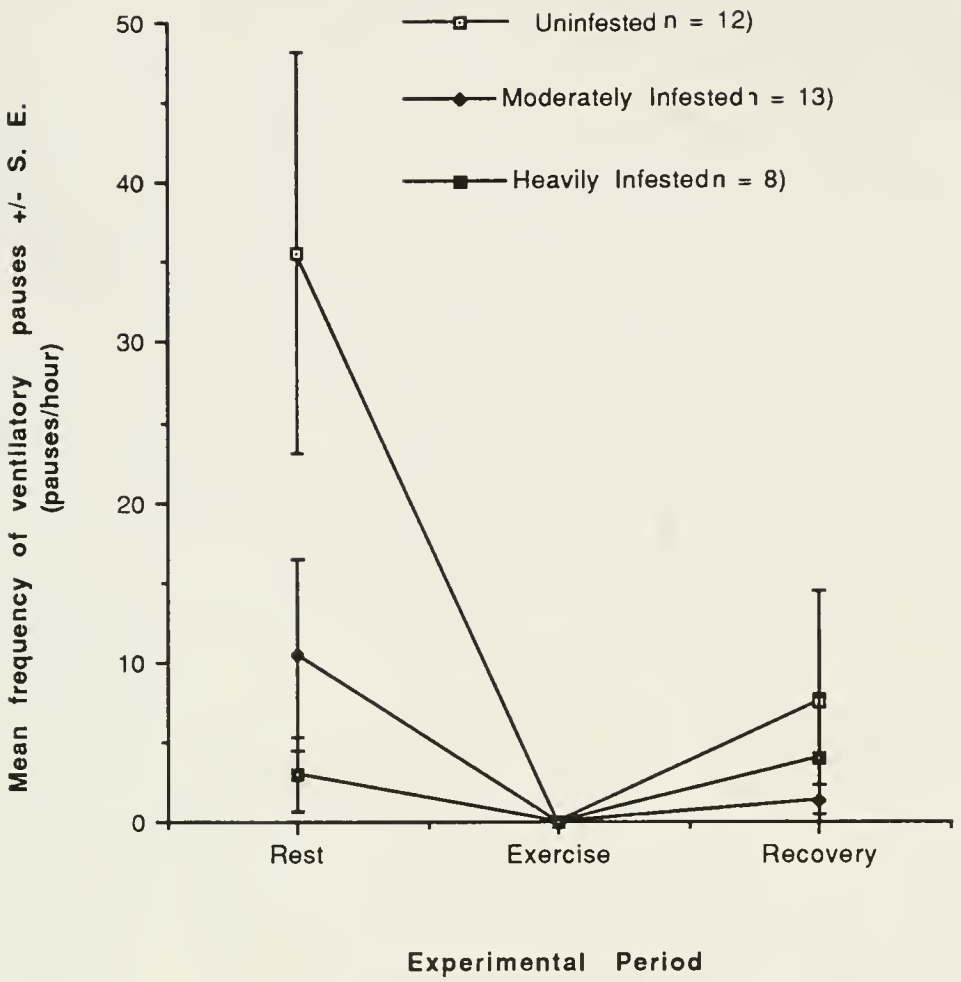


Figure 3-3. Frequency of pauses in ventilation of blue crabs at rest, exercise and recovery at three levels of infestation

Figure 3-4. Mean post- (a) and prebranchial (v) hemolymph oxygen contents of crabs at rest, exercise and recovery, at three levels of infestation. Dashed lines indicate significant ($P < 0.05$) a-v differences. Asterisk indicates an a-v difference that is significantly greater ($P < 0.05$) than for other infestation levels.

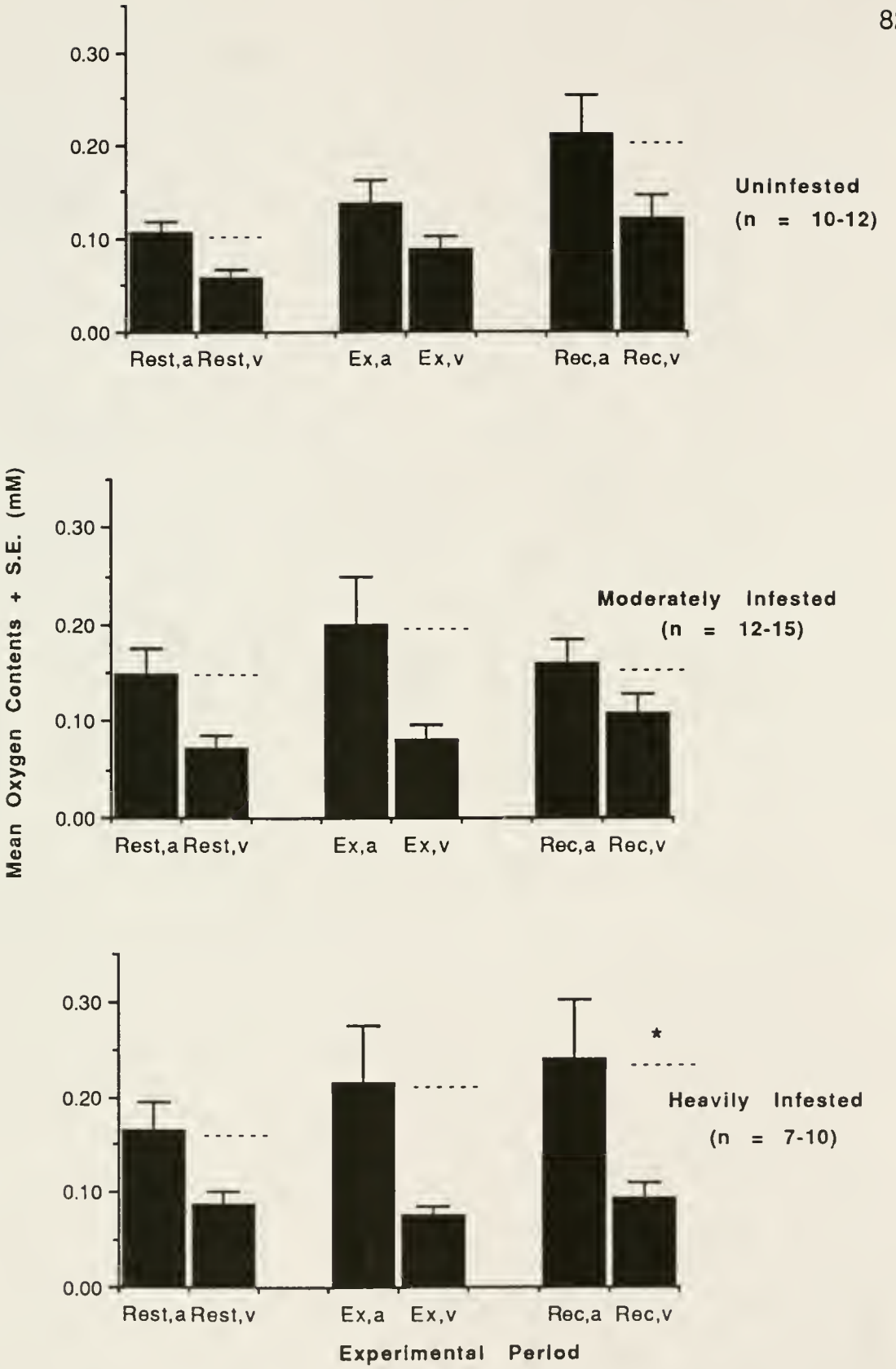

0.30
0.20
0.10
0.00
Rest,a Rest,v Ex,a Ex,v Rec,a Rec,v
Uninfested
(n = 10-12)
Moderately Infested
(n = 12-15)
Heavily Infested
(n = 7-10)
*
Mean Oxygen Contents + S.E. (mM)
Experimental Period

Figure 3-5. Mean post- (a) and prebranchial (v) hemolymph oxygen tension of crabs at rest, exercise and recovery, at three levels of infestation. The dashed line indicates a significant ($P < 0.05$) a-v difference. The asterisk indicates an a-v difference that is significantly greater ($P < 0.05$) than for other infestation levels.

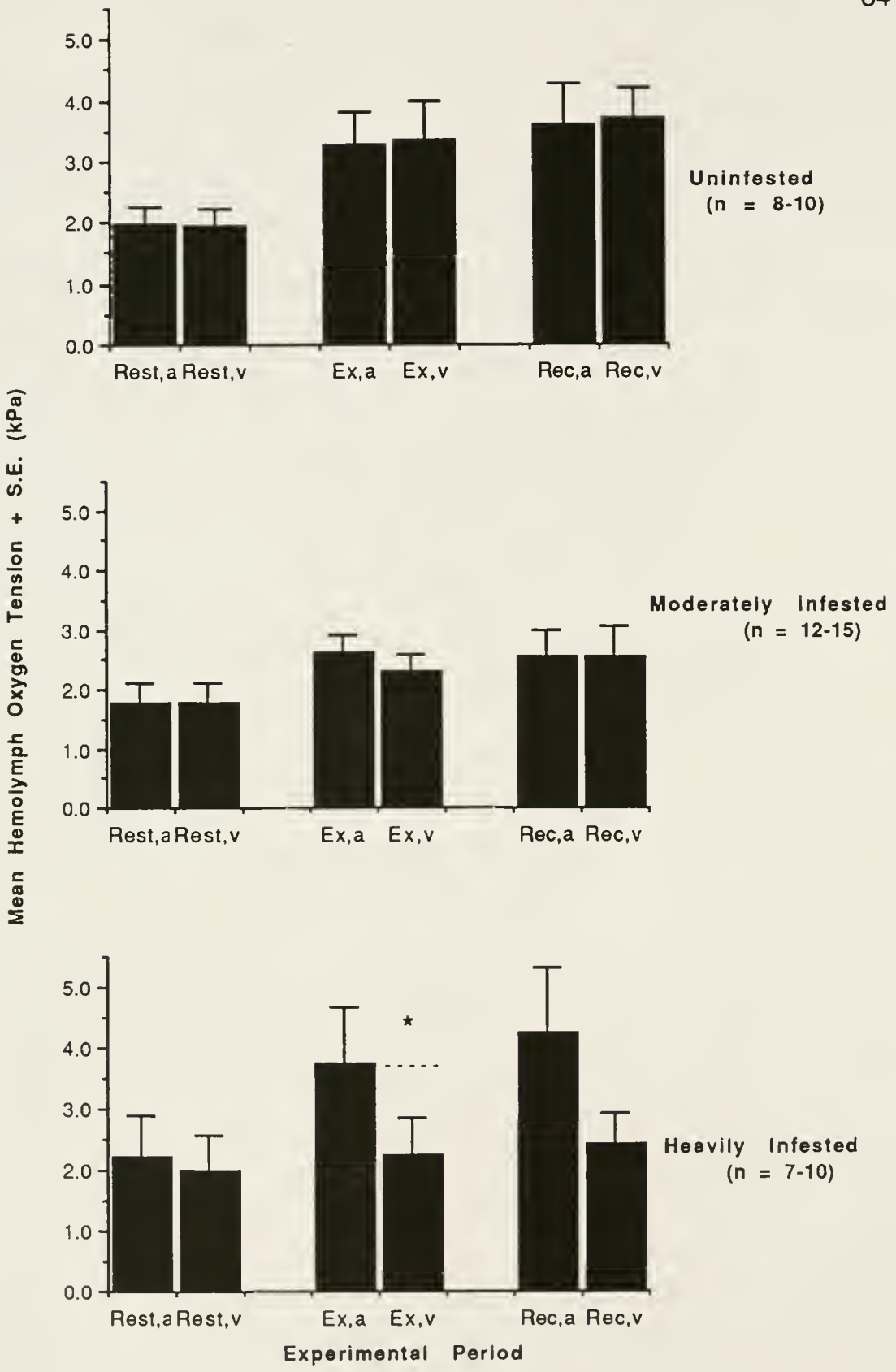

Mean Hemolymph Oxygen Tension + S.E. (kPa)
5.0
4.0
3.0
2.0
1.0
0.0
Rest,a Rest,v Ex,a Ex,v Rec,a Rec,v
Uninfested
(n = 8-10)
Moderately infested
(n = 12-15)
Heavily infested
(n = 7-10)
*
Experimental Period

Figure 3-6. Mean post- (a) and prebranchial (v) hemolymph pH of crabs at rest, exercise and recovery, at three levels of infestation. Dashed lines indicate significant ($P < 0.05$) a-v differences.

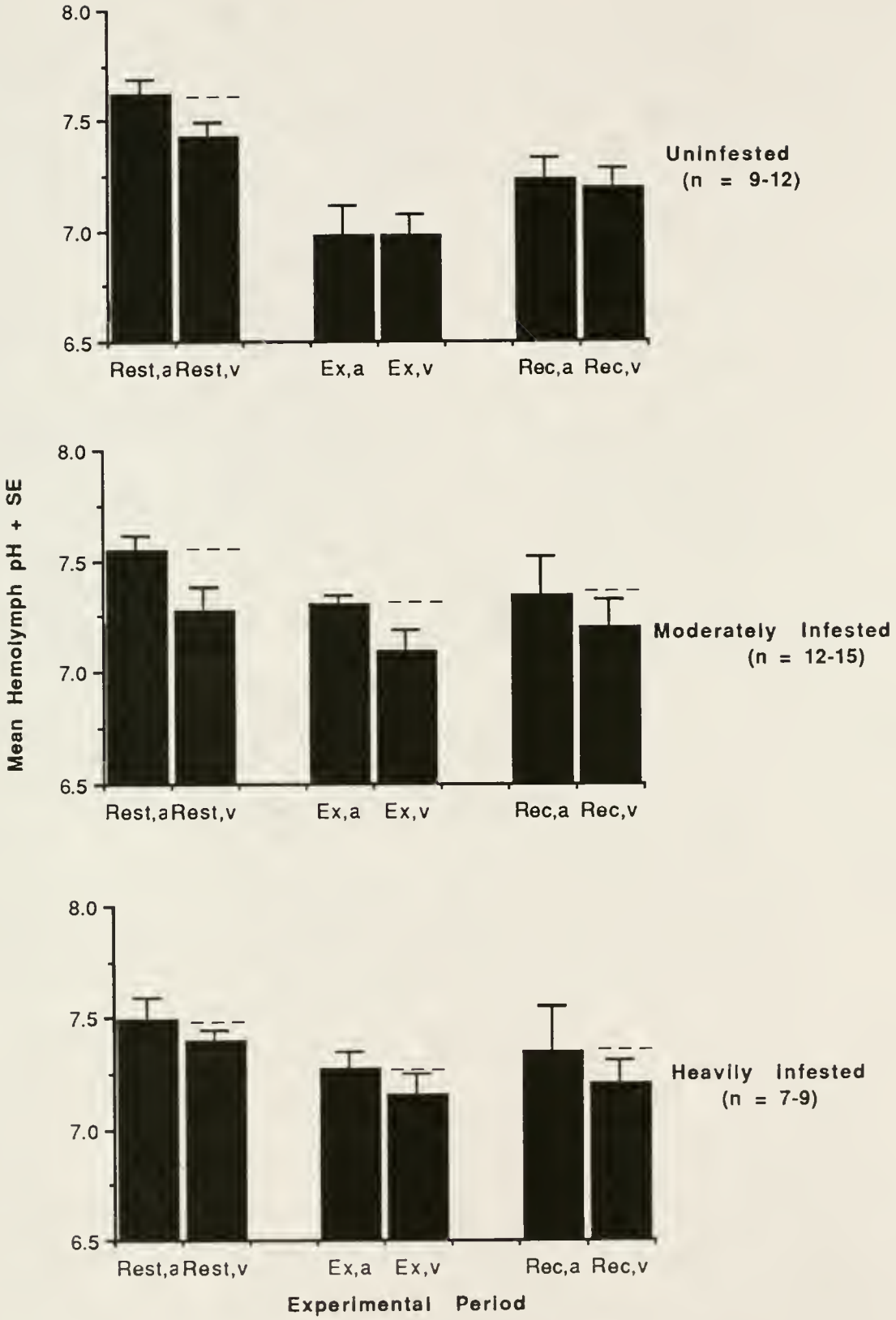
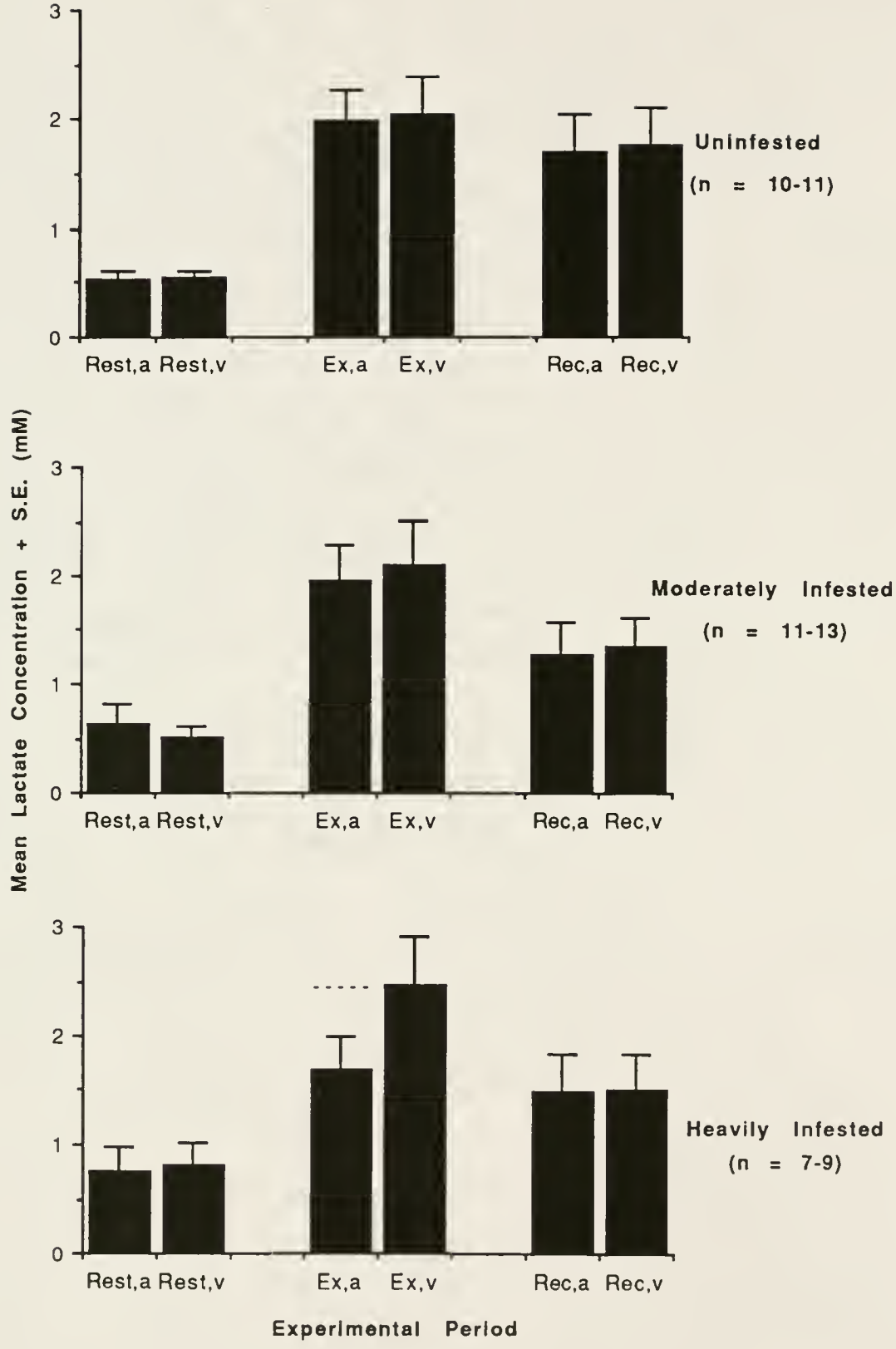

8.0
7.5
7.0
6.5
Rest,a Rest,v Ex,a Ex,v Rec,a Rec,v
Uninfested
(n = 9-12)
Moderately Infested
(n = 12-15)
Heavily Infested
(n = 7-9)
Mean Hemolymph pH + SE
Experimental Period

Figure 3-7. Mean post- (a) and prebranchial (v) hemolymph lactate concentration of crabs at rest, exercise and recovery, at three levels of infestation. The dashed line indicates a significant ($P < 0.05$) a-v difference.


Mean Lactate Concentration + S.E. (mM)
3
2
1
0
Rest,a Rest,v Ex,a Ex,v Rec,a Rec,v
Uninfested
(n = 10-11)
Moderately Infested
(n = 11-13)
Heavily Infested
(n = 7-9)
Experimental Period

GENERAL DISCUSSION

This study has focused on the Octolasmis muelleri/Callinectes sapidus relationship seeking answers to questions on three levels. On the organism level, information was collected to evaluate the likelihood that barnacle larvae select optimal sites and hosts and to determine the crab's physiological response/compensation to the barnacles presence. The optimal site and host are those that allow the highest rate of growth, survival and reproduction. On the population level, infestation rates were measured to determine if the barnacle population was large enough to have a major impact on the Cedar Key, FL crab population. On the host/ectocommensal level, information from the other two levels was synthesized to evaluate the selection pressures on the host and symbiont and to determine how this system differs from other host/symbiont systems.

Organism

Octolasmis muelleri adults aggregated on the bases of the hypobranchial aspect of gills 3,4,5 and 6, the best ventilated part of the gill chamber (Hughes et al., 1969). This represents only a small portion of the available attachment area on the gills (28.6%), and an

even smaller portion (5.7%) of the total suitable area. The suitable area consists of the areas where apparently healthy adult barnacles were found. This includes both sides of the gills, the branchial chamber epidermal lining, and the carapace underneath the gills. Although this suggests site selection, three pertinent assumptions must be evaluated: 1. the best ventilated site corresponds to the optimal site. 2. adult distribution reflects larval settlement rather than post-settlement mortality. 3. cyprid settlement involves active selection, not passive distribution.

Ventilation and the Optimal Site

The requirements of adult barnacles have not been researched in detail but must include a minimal level of oxygen (Barnes and Barnes, 1964), nutrient availability, and the presence of prospective mates. As for the latter requirement, aggregation of the adults as demonstrated in Chapter one should suffice. Barnacles are uncommon among sessile animals in that they have internal fertilization. Adult O. muelleri are able to extend their muscular stalk and obtain a length up to 2.0 cm (personal observation). Coupled with a long protrusible penis, they should be able to mate with other barnacles within a radius comprising about the area in which they were found to aggregate, but not reaching the whole gill chamber.

With respect to oxygen availability, levels are relatively high in all parts of the gill chamber but presumably the best ventilated site, would be where water first enters the gill chamber (Hughes et al., 1969). Since the blue crab takes only 50% of the oxygen out of

water passing through the gill chamber at rest (Booth et al., 1982), oxygen availability, is probably adequate,.even in the epibranchial chamber.

In regard to nutrient requirement, little information is available but adult blue crabs are omnivorous, cannibalistic, and detritivorous (Laughlin, 1982) as well as being sloppy eaters. They shred their food using the maxillipeds and mandibles before ingesting it (Pyle and Cronin, 1950), often creating a cloud of particulate matter (personal observation). The intake to their gill chamber, the Milne-Edwards opening, is at the base of the cheliped (McMahon and Wilkens, 1983) and therefore in close proximity to the mouth. Injection of methylene blue dye into the water near the base of the cheliped showed that particulate matter is swept into the gill chamber (personal observation). The diet of Q. muelleri adults in nature is unknown but they have been maintained for several months in lab cultures on crushed Artemia (Lang, 1976) which is nutritionally similar to the crustacean food components that make up a large portion of blue crab gut contents (Darnall, 1959). Therefore the best ventilated site may also be the optimal attachment site with regards to nutrient availability.

With respect to negative impacts, Q. muelleri is remarkably sheltered from predation in the blue crab gill chamber. Little is known of the relative efficiency of the blue crab gill rakers (epipodites of second and third maxillipeds (Pyle and Cronin, 1950)), which clean the surface of the gills. However, it appears that Q. muelleri avoids removal by attaching to the margins and bases of the

gills rather than directly on the efferent vessel (Walker, 1974). This would also favor the proposed optimal site.

Adult Distribution and Larval Site Selection

Differential mortality at sites within the gill chamber could cause adult distribution to be nonrepresentative of barnacle cyprid attachment site selection. Although this question has not been addressed directly, previous studies of O. muelleri distribution that included unmetamorphosed attached cyprids, did not report a difference between cyprid and adult distribution (Walker, 1974; Jeffries et al., 1983).

Active Selection Rather Than Passive Distribution

The aggregated distribution of barnacles at the best ventilated site in the gill chamber could be explained by passive distribution in the ventilatory stream and immediate attachment of larvae to the first surface they encounter. Barnacle cyprid larvae respond to surface contour (Wethey, 1986), water flow patterns (Rittschof, Branscomb and Costlow, 1984), and chemical cues (Larman, Gabbot and East, 1982) when selecting attachment sites. Cyprids are able to move against currents by swimming in the slow-moving boundary layer (Crisp, 1955) or by crawling on the surface (Crisp and Barnes, 1954). Symbiotic barnacle larvae are thought to use substrate texture cues as well as host chemical cues in settlement (Lewis, 1978). Extracts of host tissues promote settlement in several symbiotic cirripedes (Crisp and Williams, 1960; Williams, G. B., 1964; Hayward and Harvey, 1974). The less likely it is that a

settling cyprid will encounter its future habitat by random, the greater is the necessity for a mechanism of recognition (Lewis, 1978). Cyprids of a symbiotic barnacle such as O. muelleri are unlikely to encounter their specialized attachment site at random. The capability of cyprids to respond to host chemical cues has been shown for Octolasmis cor (Jeffries et al., 1989) and other symbiotic species (Crisp and Williams, 1960).

However, in the present system, the optimal site is the best ventilated site, so passive settlement and optimal site selection cannot be distinguished because they predict similar distributions. Active versus passive settlement could be distinguished with evidence that O. muelleri cyprids sample multiple sites before attaching. Such evidence could be obtained with microcinematography studies. In other studies, crabs have had sections of their carapace replaced with transparent plastic to observe ventilatory currents (Hughes et al., 1969). This preparation would work in the present case except that almost all settlement occurs in the hypobranchial chamber (See Chapter 1), and only the epibranchial chamber can be viewed easily. Until the mechanism of passive settlement is disproved, optimal site selection for O. muelleri larvae cannot be unequivocally accepted.

Host selection was suggested by the prevalence of infestation in the predicted optimal host, previously infested large adult crabs. Such crabs would provide potential mates, molt infrequently and provide a greater ventilation volume. Walker (1974) states without providing evidence that O. muelleri cyprids will attach to crabs of any age, however, in my experience they are rarely found in

immature crabs. Development of the related Q. cor from metamorphosis to sexual maturity takes only 2 weeks (Jeffries et al., 1985). However, mating, reproduction and brooding of barnacle larvae take unknown amounts of time. Development in Q. muelleri may not be as rapid as in its tropical congener. Since juvenile blue crab intermolt periods range from 2 to 4 weeks after instar 8 (Millikin and Williams, 1984), they might make suitable hosts. However, adult Q. muelleri can live and continue to reproduce for several months in an adult blue crab, therefore settlement in a juvenile crab would be suboptimal. Jeffries et al. (1989) demonstrated that Q. cor and Q. angulata cyprids can differentiate between premolt and intermolt crabs, and will wait for ecdysis to occur before attachment. Although the mechanism enabling them to do this is unknown, it is likely chemical. Similar chemical cues could be used to differentiate juvenile blue crabs from adults and could explain the 40% infestation level in adult blue crabs found in this study, compared to the < 1% infestation level in juvenile blue crabs that I have observed.

The organism level of this investigation also included the response/compensation of the infested crab. At all but the heaviest infestation levels (those crabs that died from experimental stress) and at the exercise levels used, barnacle infestation induced only a mild disturbance. The greater incidence of mortality in infested crabs during aerial exposure (2 hours) and transport from the sampling site (Gannon, 1988), and the deaths of the extremely heavily infested crabs due to experimental stress (Chapter 2) suggest that the crab's ability to compensate is limited and the

appropriate stress probably can aggravate the effects of infestation. The median infestation level (4 O. muelleri per crab) is well below these limits. The stress of sustained swimming in the blue crab is relatively minor due to the counteracting effects of lactate and H^+ on the oxygen combining characteristics of hemocyanin (Booth et al., 1984), the use of the external medium as a proton sink (Cameron, 1985; Milligan et al., 1989), and the well developed ventilatory and circulatory response (Booth et al., 1982; McMahon and Wilkes, 1989). Perhaps a different experimental stress such as aerial exposure (Batterton and Cameron, 1978) or rapid escape swimming (Spirito, 1972) would reveal a greater effect of barnacle infestation. However, the ability of the blue crab to compensate for these stresses might prove to be equal to that for sustained swimming.

The compensatory responses to barnacle infestation in resting blue crabs were hyperventilation and tachycardia. The response was minor relative to the compensatory responses to exercise (Booth et al., 1982; Milligan et al., 1989), hypercapnia and hypoxia (Batterton and Cameron, 1978).

Because barnacles were found aggregated in large mature crabs, the possibility exists that the compensatory response seen is not an effect of infestation, but rather a property of large mature crabs. However the control crabs in these physiological studies (Chapters 2 and 3) were also large mature crabs. The greater the elapsed time since a crab's last molt, the more likely it is to be infested with O. muelleri or ectocommensals of any type (Walker, 1974) Hyperventilation and tachycardia may be characteristic of crabs that are long-term postmolt. The elevated heart rates

reported in this study however are greater than those found in several studies of mature intermolt crabs (Batterton and Cameron, 1978; Booth et al., 1982;). Although it is not possible to determine the postmolt duration in a wild-caught crab, other researchers have used degree of carapace wear and presence of algae and ectocommensals as rough indicators of postmolt duration (Jeffries and Voris, 1983). In my experience, these characteristics do not seem to be accurate predictors of O. muelleri infestation in Seahorse Key blue crabs.

That the compensatory response observed is due only to the presence of the barnacle could only be tested by experimentally infesting crabs immediately after molting, and measuring their gas exchange parameters relative to uninfested immediately postmolt crabs.

Experimental infestations would allow other aspects of the association to be studied. Excision of the gill rakers before experimental infestation would allow determination of their role in preventing ectocommensal establishment. Experimental infestation would also allow measurement of barnacle development at different sites within the gill chamber, possibly confirming the designation of the base of the middle gills as the optimal attachment site.

Population

Octolasmis muelleri is a common ectocommensal in Seahorse Key blue crabs (40% overall mean infestation rate) at rates

comparable to Louisiana blue crabs (Humes, 1941). Median infestation levels (4 barnacles per crab) indicate that most of the blue crab population is not significantly affected. The difference between median and mean infestation levels (21.8 barnacles/crab) results from a small number of massive infestations (up to 482 barnacles) that probably do debilitate their hosts.

Host/Ectocommensal

Because the majority of crab hosts are not significantly disturbed, at least in terms of the measured physiological parameters, this symbiotic relationship appears to be a true commensalism. Selection pressure on the barnacle is likely to minimize impact on the host, because if the host dies the barnacle is unlikely to survive (Walker, 1974). There is also selection pressure on the crab, as host, to compensate for barnacle presence such that disturbance is minimal, because a debilitated host will be less successful in survival and reproduction. Since both parties face similar selective pressure, it is not surprising that the relationship has evolved so that the impact of infestation is minimal.

Once the barnacle attaches to the host it becomes dependent on it. Although minimizing its impact on the host, the barnacle also faces selection pressure to maximize the benefits it receives from the host. One way is to increase the rate of water the host passes through the gill chamber. This provides an increased supply of nutrients, oxygen and perhaps more potential mates as well as

increased elimination of wastes. Although a mechanism is unclear, this type of modification of host behavior and physiology, which is termed "host regulation", is common in insect parasitoids (Vinson and Iwantsch, 1980). Parasitoidic relationships evolve towards host regulation because the host is castrated by the parasitoid (Vinson and Iwantsch, 1980). Natural selection acting on a nonreproductive animal cannot cause evolutionary change. Therefore selection is incapable of enabling the host to deter parasitoid control of it. Although this is not wholly applicable to the present case, the hyperventilation (1.8 X normal rates) observed in infested crabs is disproportionate to the crabs compensatory response in other parameters. It would be difficult to demonstrate ectocommensal stimulation of hyperventilation beyond that needed for compensation without a specific mechanism, but the possibility of host regulation is intriguing and deserves further study.

Selection pressure on the crab host would be towards minimizing disturbance due to the ectocommensal. In the event that the infestation level increased to threatening levels the crab's defense would be to molt and shed the ectocommensal barnacles with the exuviae (Walker, 1974).

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BIOGRAPHICAL SKETCH

Andrew Thomas Gannon was born in Oklahoma City on June 27, 1958. He lived in the Azores, New York, Pennsylvania, the Phillipines, and New Jersey before moving to the more hospitable climate of Florida. He graduated from Largo High School in Largo, Florida, in 1976. He graduated from the University of South Florida in 1980 with a Bachelor of Arts in zoology. After working on a construction crew that built churches he entered graduate school in zoology at the University of Florida. He received a Master of Science degree from the Department of Zoology in 1986. He also received certification as an instructor of SCUBA through the UF Division of Continuing Education and as a second degree black belt through the UF Cuong Nhu Karate Club. He has taught both SCUBA and karate at UF. He met Francesca Gross in a graduate course in the zoology department (community ecology) and married her on November 11, 1989. He is a member of the American Society of Zoologists, the Crustacean Society, the Southeastern Estuarine Research Society, the Relicts of the Pleistocene, and Sigma Xi. He intends to begin a postdoctoral research position at the University of Massachusetts upon completing the requirements for the Doctor of Philosophy.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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